Niels Olsen Saraiva Camara
Jose C Alves-Filho
Pedro Manoel Mendes de Moraes-Vieira
Vinicius Andrade-Oliveira *Editors*

Essential Aspects of Immunometabolism in Health and Disease

Springer

Essential Aspects of Immunometabolism in Health and Disease

Niels Olsen Saraiva Camara · Jose C Alves-Filho ·
Pedro Manoel Mendes de Moraes-Vieira ·
Vinicius Andrade-Oliveira
Editors

Essential Aspects of Immunometabolism in Health and Disease

Springer

Editors
Niels Olsen Saraiva Camara
Laboratory of Transplantation Immunobiology
Department of Immunology
Institute of Biomedical Sciences IV
University of São Paulo
São Paulo, Brazil

Pedro Manoel Mendes de Moraes-Vieira
Laboratory of Immunometabolism
Department of Genetics, Evolution Microbiology, and Immunology
Institute of Biology
University of Campinas
Campinas, São Paulo, Brazil

Jose C Alves-Filho
Department of Pharmacology
University of São Paulo
São Paulo, Brazil

Vinicius Andrade-Oliveira
Bernardo's Lab
Center for Natural and Human Sciences
Federal University of ABC
São Paulo, Brazil

ISBN 978-3-030-86683-9 ISBN 978-3-030-86684-6 (eBook)
https://doi.org/10.1007/978-3-030-86684-6

This Springer imprint is published by the registered company Springer Nature Switzerland AG
The registered company address is: Gewerbestrasse 11, 6330 Cham, Switzerland

Preface

Immunometabolism has emerged as an intersectional crossroad between metabolism and immune response. Over the past decade, it has become clear that most, if not all, of the immune cell function is undissociated from their cellular metabolism. Although seminal works have addressed the metabolic fate of immune cells during differentiation and function, the physiological status of given tissue is also critically dependent on the cell metabolism.

The dialogue between immune cells and the microenvironment modulates the cellular metabolism that can trigger the onset as well as the progression of a multitude of inflammatory-mediated diseases. Thus, uncovering the specification of the metabolism of the different immune cells types in different conditions can shed light into the development of new drugs and therapeutical strategies to treat immune-mediated diseases.

The book *Essential Aspects of Immunometabolism* was thought to give to the readers a broader view of the different angles of how metabolic pathways can influence the several types of immune cells during activation, differentiation, and function during homeostasis and diseases. Of note, the structure of the book was created thinking not only for the immunologist but also for undergraduate and graduate students, physicians, and scientific community interested in the field. We begin in chapter "Overview of Immune Responses" with an overview of the immune response, describing the components of the system and its dynamic. Chapter "Metabolic Pathways and Cell Signaling" depicts the different routes of the metabolic pathways. This section nicely explains the metabolic pathways by describing how nutrient-sensing machinery for carbohydrate, lipid, and amino acid work and how they fuel cellular metabolism. Chapter "Mitochondrial Biogenesis and Dynamics in Health and Disease" complements and dives deeper into mitochondria metabolism and describes the main transcription factors responsible for mitochondria biogenesis. The text brings to our attention the mechanisms underlying mitochondria dynamics such as fission and fusion, and how these processes are regulated and how they impact health and disease. Chapters "Metabolic Pathways in Immune Cells Commitment and Fate" "Metabolic Profile of Innate Immune Cells" and "Metabolic Profile of Adaptive Immune Cells" specify the importance of metabolic pathways in different immune cells' activation, differentiation, function, and fate. These chapters give a

broad overview of how metabolic pathways influence innate and adaptive immune cells. Moreover, it is explained how the metabolic profile of innate immune cells in different situations is such as during phagocytosis versus efferocytosis. They also explain the different metabolic profiles in macrophages subsets, ILCs, and subtypes of dendritic cells. Finally, the metabolic profile of adaptive cells, giving the very time-frame of the metabolism of T and B cells, exactly matched with the course of adaptive immune response ranging from the ontogeny, migration, and contraction phases until memory was detailed. From that, the book starts covering the role of immune cells and metabolism in different disease conditions. The interconnection between inflammation, obesity, and metabolic syndrome, bringing different factors such as adipokines, the microbiota composition can influence adipose tissue, consequently inflammation in adipose tissue and metabolic syndrome is fully discussed. Chapter "Metabolic Reprogramming and Infectious Diseases" paves how metabolic pathways are modulated during infectious diseases, by explaining the importance of barrier tissue for homeostasis and how the metabolism of immune cells behaves facing different types of pathogens. In chapter "Metabolic Reprogramming and Cancer", the authors elegantly explain how the metabolism of cancer cells and the pathways is triggered in the cancer microenvironment. Interestingly, most of the metabolic pathways activated in cancer cells are shared by immune cells' metabolism. This book also brings insights into how adipocyte influences the regulation of immune cells, emphasizing how factors released by adipocytes can influence adipose-tissue-residing immune cells as well as the consequence of the adipose-tissue-residing cell activation for obesity and metabolic syndrome. Chapter "Immune Regulation of Adipose Tissue Browning" continues in immune-mediated and adipose tissue cross-talk with a deep focus on how this interaction regulates immune cells and adipocyte of the brown adipose tissue. Finally, the book ends with chapters "Physical Exercise and Metabolic Reprogramming" and "Immunometabolism and Organ Transplantation" bringing approaches to two more different conditions where immunometabolism can have an important impact. Chapter "Physical Exercise and Metabolic Reprogramming" described how different exercises influence the reprogramming metabolism of the immune cells and chapter "Immunometabolism and Organ Transplantation" covers the aspects of metabolic pathways and immune cells during the graft engraftment process from ischemia-reperfusion injury to acute and chronic transplant rejection.

The field has fortunately developed fast, and it is the great interest in the Immunology field. Thus, this book does not intend to cover all the aspects of immunometabolism but rather gets the readers an essential background to enable them follow the new reports that are coming up.

We thank all the authors that contributed to this book and hope the readers enjoy reading this book and the extent to their knowledge about how the immunometabolism impacts immune cells, homeostasis, and inflammatory diseases.

São Paulo, Brazil

Niels Olsen Saraiva Camara
Vinicius Andrade-Oliveira

Contents

Contributors

Fernando Abdulkader Department of Physiology and Biophysics, Instituto de Ciencias Biomedicas, Universidade de São Paulo, São Paulo, Brazil

Renan Willian Alves Center for Human and Natural Sciences, Federal University of ABC, Santo Andre, SP, Brazil

Vinicius Andrade-Oliveira Center for Human and Natural Sciences, Federal University of ABC, Santo Andre, SP, Brazil

H. P. Batatinha Department of Cell and Developmental Biology, University of São Paulo, São Paulo, Brazil

Lev Becker Committee On Molecular Metabolism and Nutrition, The University of Chicago, Chicago, IL, USA;
Ben May Department for Cancer Research, The University of Chicago, Chicago, IL, USA

Enrique Boccardo Instituto de Ciências Biomédicas, Universidade de São Paulo, São Paulo, Brazil

Luiz H. M. Bozi Institute of Biomedical Sciences, University of São Paulo, São Paulo, Brazil;
Dana-Faber Cancer Institute and Department of Cell Biology, Harvard Medical School, Boston, USA

Niels Olsen Saraiva Camara Laboratory of Transplantation Immunobiology, Department of Immunology, Institute of Biomedical Sciences, University of São Paulo, São Paulo, Brazil

Juliane C. Campos Institute of Biomedical Sciences, University of São Paulo, São Paulo, Brazil;
Joslin Diabetes Center and Department of Genetics, Harvard Medical School, Boston, USA

Ana Campos Codo Department of Genetics, Microbiology and Immunology, Institute of Biology, Campinas State University, Campinas, Brazil

Denise Morais da Fonseca Laboratory of Mucosal Immunology, Department of Immunology, Institute of Biomedical Sciences, University of São Paulo, São Paulo, Brazil

Eloisa Martins da Silva Paulista Medical School, Federal University of São Paulo, São Paulo, Brazil

Gustavo Gastão Davanzo Ben May Department for Cancer Research, The University of Chicago, Chicago, IL, USA;
Laboratory of Immunometabolism, Department of Genetics, Evolution, Microbiology and Immunology, Institute of Biology, University of Campinas, Campinas, Brazil

Letícia de Aquino Penteado Department of Biochemistry and Immunology, Ribeirao Preto Medical School, University of São Paulo, Ribeirao Preto, Brazil;
Department of Biological Sciences, School of Pharmaceutical Sciences, São Paulo State University, Araraquara, Brazil

Jean De Lima Laboratory of Transplantation Immunobiology, Department of Immunology, Institute of Biomedical Sciences, University of São Paulo, São Paulo, Brazil

Alexandra Ivo de Medeiros Department of Biological Sciences, School of Pharmaceutical Sciences, São Paulo State University, Araraquara, Brazil

Flávia Sardela de Miranda Instituto de Ciências Biomédicas, Universidade de São Paulo, São Paulo, Brazil

Érika Machado de Salles Laboratory of Immunology of Infectious Diseases, Department of Immunology, Institute of Biomedical Sciences, University of São Paulo, São Paulo, Brazil

Omar Domínguez-Amorocho Department of Molecular Biology, Universidad Autónoma de Madrid (UAM), Madrid, Spain;
Institute of Biomedical Sciences, University of São Paulo, São Paulo, Brazil;
Immunometabolism and Inflammation Laboratory, Centro de Biología Molecular Severo Ochoa, Cellular Communication & Inflammation Unit, Madrid, Spain;
Faculty of Sciences, Department of Molecular Biology, Universidad Autónoma de Madrid (UAM), Madrid, Spain

Lorena Doretto-Silva Center for Human and Natural Sciences, Federal University of ABC, Santo Andre, SP, Brazil

Julio C. B. Ferreira Institute of Biomedical Sciences, University of São Paulo, São Paulo, Brazil;
Department of Chemical and Systems Biology, School of Medicine, Stanford University, Stanford, USA

Alexandria Hoffman Committee On Molecular Metabolism and Nutrition, The University of Chicago, Chicago, IL, USA;

Ben May Department for Cancer Research, The University of Chicago, Chicago, IL, USA

K. Kruger Department of Exercise Physiology and Sports Therapy, Justus-Liebig-University Giessen, Giessen, Germany

Jennifer Lee Division of Endocrinology, Diabetes and Metabolism, Beth Israel Deconess Medical Center, Harvard Medical School, Boston, MA, USA

Luiz Osório Leiria Department of Biochemistry and Tissue Biology, Institute of Biology, University of Campinas, Campinas, SP, Brazil;
Department of Pharmacology, Ribeirão Preto Medical School, University of São Paulo, Ribeirão Preto-SP, Brazil

Ana Paula Lepique Instituto de Ciências Biomédicas, Universidade de São Paulo, São Paulo, Brazil

F. S. Lira Department of Physical Education, Universidade Estadual Paulista, Presidente Prudente, Brazil

María Mittelbrunn Immunometabolism and Inflammation Laboratory, Centro de Biología Molecular Severo Ochoa, Cellular Communication & Inflammation Unit, Madrid, Spain;
Faculty of Sciences, Department of Molecular Biology, Universidad Autónoma de Madrid (UAM), Madrid, Spain;
Instituto de Investigación Sanitaria Hospital, Madrid, Spain

Pedro Manoel Mendes de Moraes-Vieira Department of Genetics, Microbiology and Immunology, Institute of Biology, Campinas State University, Campinas, Brazil;
Experimental Medicine Research Cluster (EMRC), University of Campinas, São Paulo, Brazil;
Laboratory of Immunometabolism, Department of Genetics, Evolution, Microbiology and Immunology, Institute of Biology, University of Campinas, Campinas, Brazil

Marcelo A. Mori Department of Biochemistry and Tissue Biology, Institute of Biology, University of Campinas, Campinas, SP, Brazil

Bárbara Cristina Pizzolante Laboratory of Mucosal Immunology, Department of Immunology, Institute of Biomedical Sciences, University of São Paulo, São Paulo, Brazil

Andréa L. Rocha Department of Biochemistry and Tissue Biology, Institute of Biology, University of Campinas, Campinas, SP, Brazil

J. C. Rosa Neto Department of Cell and Developmental Biology, University of São Paulo, São Paulo, Brazil

Anderson Sá-Nunes Department of Immunology, Institute of Biomedical Sciences, University of São Paulo, São Paulo, SP, Brazil

Tim J. Schulz German Institute of Human Nutrition Potsdam-Rehbruecke (DIfE), Potsdam, Germany;
German Center for Diabetes Research (DZD), München-Neuherberg, Germany;
Institute of Nutritional Science, University of Potsdam, Potsdam-Rehbrücke, Nuthetal, Germany

Gonzalo Soto-Heredero Immunometabolism and Inflammation Laboratory, Centro de Biología Molecular Severo Ochoa, Cellular Communication & Inflammation Unit, Madrid, Spain;
Faculty of Sciences, Department of Molecular Biology, Universidad Autónoma de Madrid (UAM), Madrid, Spain;
Instituto de Investigación Sanitaria Hospital, Madrid, Spain

Overview of Immune Responses

Anderson Sá-Nunes

1 General Aspects of the Immune System

The immune system is classically described as the network responsible for the 'protection' of our body against foreign substances or microorganisms, infectious or non-infectious. The immune responses are usually initiated by products or constituents of bacteria, viruses, fungi, parasites, or any other substance recognized as non-self, although self-components are also able to trigger immune reactions under specific situations. Even though the 'defense' is in fact a major function, it is well known nowadays that the physiological role of the immune system is much broader and includes tissue repairment, tolerance and antitumoral immunity. All these activities depend on a process called **recognition**, that results from the interaction between soluble and cell-associated receptors—immune sensors of the microenvironment—and their respective ligands. When a receptor-ligand interaction occurs, a series of molecular signals are initiated followed by the activation of biochemical cascades that culminate with the production of effector molecules and mediators that regulate the cellular activity. The net result of these multiples interactions produces **immunity** or **tolerance** states. However, when these interactions are deregulated, they may also cause **hypersensitivity** or **autoimmune** reactions.

Didactically speaking, the most common division used to describe the immune system takes into consideration the time required to the response against an aggressor agent to be initiated. Thus, the **innate (natural, native) immunity** is responsible for the responses that occur immediately or soon after the first contact with any given microorganism or its products [24]. The cells and molecules of the innate immunity are ready to perform its functions at the time of recognition of the attacking agent, or immediately following this contact. On the other hand, the **adaptive (acquired)**

A. Sá-Nunes (✉)
Department of Immunology, Institute of Biomedical Sciences,
University of Sao Paulo, Sao Paulo, SP, Brazil
e-mail: sanunes@usp.br

N. O. S. Camara et al. (eds.), *Essential Aspects of Immunometabolism in Health and Disease*, https://doi.org/10.1007/978-3-030-86684-6_1

immunity takes a few days to be fully developed and its effector mechanisms are mediated by cells called lymphocytes and their products [12]. In some cases, both lymphocytes and the molecules they produce are able to act directly on an extracellular microorganism or a cell infected with an intracellular microorganism. In other situations, lymphocyte-derived products amplify the effector mechanisms of the already existing innate immunity.

In this initial chapter, the reader will be presented to an overview of the cellular components of the immune system and the properties of innate and adaptive responses. It is important to make clear that, for obvious reasons, this text presents a short overview of the knowledge from immunology textbooks but does not replace them. Consequently, it is strongly recommended to the reader that, before reading this book, fundamental concepts on how the immune system works should be attained.

2 Cells of the Immune System

Before further development on the effector mechanisms of the innate and adaptive immune responses, we will start with a general overview on the major cells that constitute the immune system, their production in the bone marrow, release to the blood and, finally, migration to the tissues.

Hematopoiesis—the formation of blood cellular components—begins at early stages of the embryonic development, initially in the yolk sac (mesoblastic phase) and later in the fetal liver and spleen (hepatic phase). Around halfway of the gestation period, the bone marrow gradually assumes the blood production and becomes the exclusive hematopoietic organ by the individual birth (medullary phase), although sites of extramedullary hematopoiesis are recognized in some situations. In the first years after birth, the marrow of essentially every bone produces blood cells. However, along the body's development, this activity becomes restrict to the flat bones (e.g. sternum, pelvis) and some long bones (e.g. femur) [18].

The **hematopoietic stem cells** are multipotent and self-renewing bone marrow cells that generate all the cells in the blood, including those related to the immune system [23]. The hematopoietic stem cells are located in anatomic sites of the bone marrow associated with stromal cells, which do not present hematopoietic activity, but provide the biochemical and the contact-dependent signals required to the proliferation and differentiation of the hematopoietic stem cells. The biochemical signals are represented by growth factors from the **cytokine** family, a group of soluble molecules responsible for the 'communication' of the immune system [11]. In addition to being involved in hematopoiesis, the cytokines have essential roles in the innate and adaptive immune responses including the inflammatory process [20]. Further details will be later discussed in the chapter.

Briefly, the hematopoietic stem cells constantly divide by a process of asymmetric division producing two daughter cells. While one of the daughter cells preserves self-renewal features, the other initiates a differentiation process and develops into a **hematopoietic progenitor**. Along their development, the hematopoietic progenitors

are exposed to certain patterns of cytokines and other growth factors and further differentiate either in **myeloid** or **lymphoid precursors**. The myeloid precursors will give rise to monocytes, neutrophils, eosinophils, basophil, erythrocytes (red blood cells) and megakaryocytes (that will later generate platelets). The lymphoid precursors will give rise to T lymphocytes, B lymphocytes, natural killer (NK) cells and, possibly, some dendritic cells. In a steady-state condition, some of these cells will remain in the blood stream during their whole life (hours to days), unless the organism faces inflammatory and/or infectious challenges; other cells naturally migrate to the different tissues where they will constitute the **resident cells**; and some others will recirculate between blood and lymph and their respective draining organs, spleen and lymph nodes. Each one of these cell populations display a characteristic set of cell surface markers called **cluster of differentiation** (CD) employed to phenotype subpopulations/subsets, differentiation stages, degree of activation and metabolic states [5]. Together, these markers enable us to study different cells in vitro and in vivo and allow comparisons of experimental assays between different research groups.

3 Innate Immunity

A number of physical, chemical and biological barriers constitute the innate immunity and protects our body from noxious agents found in the environment. Among these barriers are the epithelial and mucosal surfaces, the airflow resulting from breathing, coughing and sneezing, the flow of fluids generated by lacrimation and urination, bodily secretions that possess a typical pH and set of enzymes, secretion of mucus and antimicrobial molecules and, finally, the microbiome present in all body's surfaces [21]. Each of these elements has a special role in maintaining the organism's homeostasis, but they are not always sufficient to keep the organism free from aggressive agents. When these initial barriers are "broken" and our body is invaded by a microorganism (pathogenic or not) or another type of aggressor, the innate immune system recognizes conserved structures of these organisms and endogenous molecules produced or released as a result of this invasion. Thus, when **pathogen-associated molecular patterns** (PAMPs) or **damage/danger-associated molecular patterns** (DAMPs) are directly recognized by soluble or cell-associated **pattern recognition receptors** (PRRs), a series of innate immunity events are triggered [26].

Nucleic acids, proteins, lipids and carbohydrates are examples of PAMPs exclusively or differentially expressed by groups of microorganisms that signal their presence to the immune system. DAMPs, on the other hand, are products derived from cellular damage caused by infection or a result of sterile injuries caused by insufficient blood supply and by the action of chemical and physical agents such as toxins, burns, and trauma, among others [3]. In turn, PRRs present in the blood, body fluids and also associated with cells (in vesicles, in the cytoplasm or in the cell surface) recognize these PAMPs and DAMPs and promote their neutralization and removal, either

through uptake by cells known as **phagocytes** or by activation of cytotoxic mechanisms dependent on other cell types and the activation of biochemical cascades. As this is a very broad topic, more details about PAMPs and DAMPs, as well as their molecular recognition by PRRs, can be found in immunology and biochemistry scientific manuscripts [14, 27]. Among the cells of the immune system that participate in innate immunity, we highlight the **alarm cells**, **professional phagocytes** and **cytotoxic cells**. A brief summary of each category is provided below:

- **Alarm cells** are tissue-resident cells capable of directly recognizing aggressor agents and their products. Alarm cells have either preformed mediators or produce them shortly after activation, triggering tissue inflammation. Once the inflammatory process is triggered, changes in the microcirculation occurs, leading to the extravasation of soluble molecules that mediate the initial responses against these agents associated with the recruitment of inflammatory cells from the blood circulation. Tissue-resident mast cells, eosinophils and macrophages are examples of alarm cells.
- **Professional phagocytes** are cells whose main function is to engulf microorganisms, particles and other substances by phagocytosis or endocytosis, leading to their destruction. They may be resident cells (e.g.,: macrophages and dendritic cells) or recruited to the tissues during the inflammatory process (e.g.,: neutrophils and inflammatory monocytes/macrophages).
- **Cytotoxic cells** present cytotoxic granules in their cytoplasm that can be released by exocytosis upon recognition of certain group of ligands in the surface of target cells. Cytotoxic cells are able to kill other cells infected by intracellular bacteria, viruses or cells that undergo malignant transformation and that can originate tumors. The best-known cytotoxic cells of the innate immunity are "natural killer" (NK) cells, but other cells such as macrophages, neutrophils and eosinophils can become cytotoxic in certain situations.

In addition to cellular components, the innate immunity also comprises soluble mediators. The recognition of offending agents can trigger the rapid production and release of **acute-phase proteins**, **inflammatory cytokines**, **chemotactic agents** (molecules that recruit cells to the site) and **vasoactive amines** (substances that activate the vascular endothelium), in addition to the activation of biochemical cascades responsible for blood clotting and the **complement system**, whose components promotes direct lysis of microorganisms.

As said before, once the activation of innate immunity occurs either due to an infectious agent or injury by a physical, chemical or biological agent, its elements will initiate the so-called inflammatory process. Inflammation is a process that involves local vascular changes in the microcirculation such as vasodilation and increased vascular permeability, but that may progress to more serious systemic reactions. The inflammatory process is mediated by the cellular and soluble components described above, whose consequences are represented by five classic signs: **pain** (*dolor*), **heat** (*calor*), **redness** (*rubor*), **swelling** (edema or *tumor*) and **loss of function** (*functio laesa*) [17]. In this microenvironment, the elements of innate immunity work together

to contain/destroy the aggressor agent and also initiate the activation of adaptive immunity, discussed below.

For space limitations, the inflammatory process will not be described in further details, but the topic can be found in immunology, pharmacology and pathology textbooks. In addition, many immunometabolic aspects of the innate immunity and inflammation will be approached in the next chapters.

4 Adaptive Immunity

Invading microorganisms have varied strategies for evading immune responses. In many situations, the cells and soluble factors of innate immunity are not efficient in eradicating them from our body. In the first days following infection, the elements of innate immunity attempt to control the growth and spread of microorganisms. Meanwhile, the cells of adaptive immunity need to be activated so that they can fully exert their effector functions as soon as they are ready for that. The cells of the adaptive immunity are collectively called **lymphocytes** and will be responsible, either directly or indirectly, for the effector mechanisms against the different aggressor agents. There are two major populations of lymphocytes, known as **T cells** (or T lymphocytes) and **B cells** (or B lymphocytes). T cells were named after the discovery that the **thymus** is the organ responsible for their maturation and selection ("T" from thymus) [15]. Several subpopulations of T cells are currently known that, together, represent the **cell-mediated adaptive responses** described in more details later. Likewise, B cells were described following the observation that chickens whose bursa of Fabricius had been removed were not able to produce **antibodies** [4]. The importance of antibodies as the effector molecules of **humoral adaptive immune responses** was already known, but the role of B cells in their production was uncovered by these studies ("B" from bursa). The structure and role of antibodies will be also discussed below. Humans do not have bursa of Fabricius, and our B cells are produced, matured and selected in the bone marrow (although the spleen also participates of the process). Because the bone marrow, bursa of Fabricius and the thymus are the primary sites responsible for the production and development of lymphocytes, these organs are collectively called **primary** (or **central**) **lymphoid organs**.

Lymphocytes express receptors with high specificity and accessory molecules on their surface that allow their activation and function as markers used by researchers to characterize different lymphocyte populations and subpopulations. The lymphocyte receptors are able to recognize a very large number of microbial and non-microbial substances collectively called **antigens**. For example, **T cell receptors** (TCRs) recognize peptides resulting from the processing of extracellular or intracellular proteins, derived from our own organism or from microorganisms and their products. The processing of such proteins takes place in specialized cells known as professional **antigen-presenting cells** (APCs) which are capable of capturing, degrading and displaying fragments of these proteins on the surface linked to molecules of the **major histocompatibility complex** (MHC). There are three types of professional

APC in our organism: dendritic cells, macrophages and B cells. In summary, the T lymphocyte can only be activated when its TCR (together with accessory molecules) recognizes peptides displayed by the MHC in the APC surface [10]. On the other hand, **B cell receptors** (BCRs) are able to directly recognize antigens of different biochemical nature such as proteins, carbohydrates, lipids and nucleic acids, among others. The BCR is an antibody linked to the membrane of B cells that binds cell-associated or soluble antigens present in the extracellular *milieu.* The binding of antigens by the BCR delivers activation signals to B cells which, in turn, produce and secrete large quantities of the antibody in a soluble form.

Unlike innate immunity cells, which individually express a large number of PRRs (thus being able to recognize many different PAMP and DAMP at the same time), lymphocytes have their receptors distributed clonally. That is, a lymphocyte expresses hundreds of thousands of receptors on its surface, but in an individual cell (**clone**), all of these receptors recognize the same antigen. Therefore, although an individual has millions of lymphocytes in its circulation, only a few clones will be specific for a given antigen. The sum of all antigens capable of being recognized by lymphocyte clones present in our body constitutes an individual's **immune repertoire** [13]. As a consequence of the small number of specific clones for each antigen, once activated, lymphocytes must enter a process of **clonal expansion** in order to reach a critical number of cells capable of handling each antigen. Because it takes 8–12 h for a lymphocyte clone to complete each division cycle, a few days (typically 5–12 days) are needed until the role of adaptive immunity in an infection is perceived. However, the great advantage of the adaptive immunity is the generation of **immunological memory**, that is, once activated, part of the lymphocytes will become memory cells and will survive for months or years, even after the pathogen/antigen elimination [16, 25]. In subsequent contacts with the same agent, these cells will be present in greater numbers and will be activated more quickly, ensuring full and fast protection to our organism. For that reason, most pathogens only cause disease the first time they come into contact with our organism. This knowledge is widely explored in the development of **vaccines**, which are safe preparations capable of mimicking an infection, leading to the development of protective immune responses and immunological memory when we come into contact with the real pathogen [19].

The activation of lymphocytes is a complex process and results from the recognition of antigens by TCR or BCR, triggering intracellular signaling cascades responsible for the transduction of activation signals. Together, these signals induce activation of protein kinases, phosphorylation of biochemical substrates, increase in cytoplasmic calcium and activation of nuclear transcription factors that lead to cell proliferation and synthesis of molecules responsible for the effector mechanisms of T cells and B cells. Such cascades will be covered in this book, when appropriate, in the context of immunometabolism.

4.1 Cell-Mediated Adaptive Immune Responses

As mentioned before, **cell-mediated adaptive immune responses** are initiated when APCs present at the site of an infection (usually immature dendritic cells in the tissues), capture microorganisms or their products and process protein antigens for presentation by MHC molecules. The whole process is accompanied by **dendritic cell maturation**, leading to phenotypic changes and culminating in morphological and physiological changes in these cells, followed by migration to draining lymph nodes [2]. In cases where the infection occurs directly in the bloodstream, the microorganisms and their products will be drained by the spleen, where antigen processing and presentation will take place by splenic APCs. Lymph nodes, spleen and other organized lymphoid clusters known as mucosa-associated lymphoid tissue (MALT), bronchus-, larynx- and nose-associated lymphoid tissue (BALT, LALT, NALT) and gut-associated lymphoid tissue (GALT), are collectively known as **peripheral** (or **secondary**) **lymphoid organs**, and represent the places where lymphocytes accumulate during their recirculation in the body and where the activation of these cells occurs. In these lymphoid organs and tissues, newly arrived and already mature dendritic cells express high levels of MHC molecules containing peptide fragments derived from microbial proteins that will bind to TCRs, representing the **first signal of activation**. Mature dendritic cells also express costimulatory molecules that, upon interaction with their respective ligands present in T cells, will represent the **second signal of activation**. Finally, APC/T lymphocyte interactions occur in the presence of cytokines that some authors consider the **third signal of activation** [7]. Together, these molecular interactions will induce T cell proliferation and differentiation into effector cells. Once differentiated, the lymphocytes will leave the peripheral lymphoid organs/tissues and migrate to the site where the infection was originally established, thus performing its effector functions. As mentioned before, some of these cells will differentiate into memory cells.

When the antigens processed by dendritic cells are exogenous/extracellular in nature (e.g. captured by phagocytosis/endocytosis), they will be displayed by MHC class II molecules and the complex will interact with T cells that express a co-receptor called CD4. These $CD4^+$ T cells are also known as **T helper** (**Th**) cells. During the final stage of activation Th cells will differentiate into cytokine-producing cells that receive different terminologies according to the cytokine profile produced (e.g. Th1, Th2, Th17, Tfh) [8]. In turn, the cytokines produced by T cells will activate/modulate effector cells such as neutrophils, eosinophils, macrophages and B lymphocytes among others, responsible for battling and eradicating the microorganism/offending agent—toxins, poisons, bacteria, viruses, fungi, helminths, protozoa, and others. During this process, **regulatory T cells** (**Treg**) are also generated, whose role is to maintain tolerance and regulate immune responses, preventing them from becoming exacerbated or harmful [22]. On the other hand, when the antigens processed by dendritic cells are endogenous/intracellular in nature (e.g. products of viral infections or intracellular bacteria), they will be displayed by MHC class I molecules and the complex will interact with T cells that express a co-receptor called CD8. These $CD8^+$

T cells are also known as **cytotoxic** or **cytolytic T cells** (**Tc**) [9]. After activation, Tc lymphocytes will differentiate into cells capable of killing other cells infected by intracellular bacteria or viruses.

4.2 Humoral Adaptive Immune Responses

Adaptive humoral immune responses are represented by antibodies, also called immunoglobulins or gammaglobulins. Antibodies are glycoproteins produced by B cells that are capable of recognizing and binding to antigens of different biochemical nature (proteins, carbohydrates, lipids, nucleic acids, among others). In mature B cells that have never found the antigen (naïve cells), antibodies are present on the membrane surface and act as BCR for these cells. A foreign invading agent and/or its antigens will be carried to secondary lymphoid organs and will be recognized by the BCR of some B cell clones that are constantly recirculating among these organs. The BCR/antigen interaction can directly activate B cells and induce their proliferation (**clonal expansion**) and differentiation into **plasma cells**, capable of secreting large amounts of that specific antibody. These antibodies will circulate in the body fluids or will be transported to mucosal surfaces, where they remain available to bind any suitable antigen for varied periods of time. This interaction, known as the **antigen–antibody reaction**, will be essential to eliminate the offending agent directly (e.g. neutralizing toxins, bacteria, viruses, etc.) or indirectly (e.g. increasing phagocytosis, activating phagocytes and the complement system, promoting antibody-dependent cytotoxicity), as described below. It is worth mentioning that in some situations B cell can also work as APCs and be activated with the help of **T follicular helper** (**Tfh**) cells, the so-called **T-dependent** activation [6]. In other situations, the B cell can be activated directly, without the participation of Th cells, the so-called **T-independent** activation [1]. The requirements for each type of activation will not be covered in this chapter, but will depend on the population of B cells, the nature and amount of the antigen, and other factors present in the microenvironment during activation. In addition, only T-dependent activation will be able to generate memory B cells.

Briefly, the antibody consists of two identical larger chains (**heavy chains**) and two smaller identical chains (**light chains**). The heavy chains are linked to each other by disulfide bonds and each heavy chain is linked to a light chain also by disulfide bonds. Both heavy and light chains are constituted of domains: regions with a relatively conserved sequences of amino acids that repeats throughout the molecule. The heavy chains have 4 or 5 domains each (one called a variable domain and the others called constant domains), while the light chains always have 2 domains (a variable domain and a constant domain). In the region where the two domains of each light chain are paired with the respective domains of the heavy chain, there is a portion that recognizes the antigen called, therefore, **antigen-binding fragment** (**Fab**). An antibody monomer has two Fab. At the tip of each Fab there is one region formed by the outermost portions of the variable domains of heavy chain and light chain, where the interaction with the antigen effectively occurs. Therefore, this

portion is called **antigen-binding region** and concentrates most of the variability of each antibody molecule. On the other hand, the region where the two heavy chains are paired is responsible for the effector functions of the antibody. Because of its biochemical characteristics, it is called a **crystallizable fragment** (**Fc**). The region that separates the Fc from the two Fabs present in a monomeric antibody molecule is called **hinge** and gives flexibility to the molecule, thus allowing a monomeric antibody to bind to two identical antigens at the same time and at different angles. Some classes of antibodies can form dimers, trimers, pentamers and hexamers, so that they can bind multiple identical antigens at the same time (four, six, ten and twelve, respectively).

It is important to note that "antibody" is a generic nomenclature that actually represents a group of related molecules that have some structural and functional differences. According to these characteristics, antibodies are grouped into five **isotypes** (or **classes**) that in humans and other mammals: IgM, IgD, IgG, IgA and IgE. In some cases, there are subclasses (e.g. IgG1, IgG2, IgG3 and IgG4 in humans) that are differentially expressed depending on the type of immune responses triggered by a given infection. In all cases, antibodies bind to antigens in a non-covalent and reversible manner, through a series of biochemical interactions that include electrostatic forces, hydrogen bonds, van der Waals forces and hydrophobic interactions. Together, these forces define the affinity of an antibody binding site to the antigen, and the overall strength of all binding sites is called avidity (as we have seen, an antibody has at least two antigen binding sites). This bond is strong enough to ensure that, once bound, the antibody will hardly detach from its respective antigen. This ensures that antibody's effector functions can be adequately performed, including neutralization of toxins, viruses and bacteria, activation of the complement system, antibody-dependent cell cytotoxicity and opsonization (facilitation of phagocytosis).

In addition to essential effector molecules against a variety of infections, antibodies are also important for assessing the immunity developed after a vaccination and for diagnosing a range of diseases. As antibodies are present in the circulation, the collection of a small amount of blood makes it possible to assess in a patient's serum or plasma whether he/she has already been exposed to a certain antigen. In certain cases, it is even possible to determine whether the clinical condition is due to an acute or primary infection or whether the infection has already become chronic or recurrent, based both on the isotype/class and the antibody title (an indirect way of assessing the amount of antibodies present for a given antigen). On the other hand, the absence of one or more classes of antibodies may be indicative of **immunodeficiencies**.

Antibodies are among the most studied molecules in the biochemical field both structurally and functionally. This knowledge allowed the development of serum therapy over a century ago, still used nowadays to treat the bites of venomous animals such as snakes, spiders and scorpions; bacterial-derived toxins such as diphtheria and tetanus toxins; and even active infections such as Ebola and COVID-19. Advances in cell culture, establishment of tumoral cell lines and development of molecular biology techniques allowed the creation of **monoclonal antibodies**—identical antibodies with unique and known specificity—that can be produced in industrial scale

for research and clinical use. The monoclonal antibodies are the major representatives of the so-called **immunobiologicals**, molecules of biological origin capable of modulating the immune system and used as a treatment of several pathologies of microbial origin, cancer and autoimmune diseases. Immunobiologicals are already a reality for a number of clinical conditions and are among the blockbuster drugs for the pharmaceutical industry. However, its price is still high, which limits its access to most of the population.

5 Final Remarks

Now that you reviewed how immune cells are generated, how foreign agents are recognized, and how the immune responses that will fight them are initiated, the next chapters will present deeper aspects of these topics in a context of immunometabolism. You will have the opportunity to learn more details about metabolic pathways and mitochondrial physiology in health and disease. In addition, up to date knowledge on immunometabolism will be reviewed in a number of clinical conditions such as obesity, cancer, autoimmune diseases, organ transplantation and inflammatory/infectious diseases. Finally, interfaces between immunometabolism and microbiota, physical exercise, immunotherapies and translational medicine will be also approached.

References

1. Allman D, Wilmore JR, Gaudette BT (2019) The continuing story of T-cell independent antibodies. Immunol Rev 288(1):128–135
2. Austyn JM (2016) Dendritic cells in the immune system-history, lineages, tissues, tolerance, and immunity. Microbiol Spectr 4(6)
3. Bianchi ME (2007) DAMPs, PAMPs and alarmins: all we need to know about danger. J Leukoc Biol 81(1):1–5
4. Cooper MD (2015) The early history of B cells. Nat Rev Immunol 15(3):191–197
5. Cruse JM, Lewis RE, Wang H (2004) Cluster of differentiation (CD) antigens. Academic Press, Immunology Guidebook, pp 47–124
6. Crotty S (2015) A brief history of T cell help to B cells. Nat Rev Immunol 15(3):185–189
7. Curtsinger JM, Mescher MF (2010) Inflammatory cytokines as a third signal for T cell activation. Curr Opin Immunol 22(3):333–340
8. Gagliani N, Huber S (2017) Basic aspects of T helper cell differentiation. Methods Mol Biol 1514:19–30
9. Golstein P, Griffiths GM (2018) An early history of T cell-mediated cytotoxicity. Nat Rev Immunol 18(8):527–535
10. La Gruta NL, Gras S, Daley SR, Thomas PG, Rossjohn J (2018) Understanding the drivers of MHC restriction of T cell receptors. Nat Rev Immunol 18(7):467–478
11. Lin JX, Leonard WJ (2019) Fine-Tuning Cytokine Signals. Annu Rev Immunol 26(37):295–324
12. Litman GW, Rast JP, Fugmann SD (2010) The origins of vertebrate adaptive immunity. Nat Rev Immunol 10(8):543–553

13. Liu X, Wu J (2018) History, applications, and challenges of immune repertoire research. Cell Biol Toxicol 34(6):441–457
14. Medzhitov R (2009) Approaching the asymptote: 20 years later. Immunity 30(6):766–775
15. Miller JF (2004) Events that led to the discovery of T-cell development and function—a personal recollection. Tissue Antigens 63(6):509–517
16. Mueller SN, Gebhardt T, Carbone FR, Heath WR (2013) Memory T cell subsets, migration patterns, and tissue residence. Annu Rev Immunol 31:137–161
17. Nathan C (2002) Points of control in inflammation. Nature 420(6917):846–852
18. Orkin SH, Zon LI (2008) Hematopoiesis: an evolving paradigm for stem cell biology. Cell 132(4):631–644
19. Pollard AJ, Bijker EM (2021) A guide to vaccinology: from basic principles to new developments. Nat Rev Immunol 21(2):83–100
20. Ramani T, Auletta CS, Weinstock D, Mounho-Zamora B, Ryan PC, Salcedo TW, Bannish G (2015) Int J Cytokines: the good, the bad, and the deadly. Toxicol 34(4):355–65
21. Riera Romo M, Pérez-Martínez D, Castillo FC (2016) Innate immunity in vertebrates: an overview. Immunology 148(2):125–139
22. Sakaguchi S (2011) Regulatory T cells: history and perspective. Methods Mol Biol 707:3–17
23. Sawai CM, Babovic S, Upadhaya S, Knapp DJHF, Lavin Y, Lau CM, Goloborodko A, Feng J, Fujisaki J, Ding L, Mirny LA, Merad M, Eaves CJ, Reizis B (2016) Hematopoietic stem cells are the major source of multilineage hematopoiesis in adult animals. Immunity 45(3):597–609
24. Thaiss CA, Levy M, Itav S, Elinav E (2016) Integration of innate immune signaling. Trends Immunol 37(2):84–101
25. Weisel F, Shlomchik M (2017) Memory B cells of mice and humans. Annu Rev Immunol 26(35):255–284
26. Yin Q, Fu TM, Li J, Wu H (2015) Structural biology of innate immunity. Annu Rev Immunol 33:393–416
27. Zindel J, Kubes P (2020) DAMPs, PAMPs, and LAMPs in immunity and sterile inflammation. Annu Rev Pathol 24(15):493–518

Metabolic Pathways and Cell Signaling

Fernando Abdulkader

Metabolism is one of the key features of life, as it allows for the continuous rebuilding of the living structures driven by the extraction of energy from nutrients [1]. As such, metabolism can be thought of as the set of all the chemical reactions that take place in a living organism and are related to the maintenance of life. In principle, many more reactions could happen between the chemical substances that are found in living matter than the number we observe when organisms are actually alive. Those other reactions, albeit possible, would not sustain the maintenance of the living structures. So, the smaller set of reactions that do sustain life—that is, metabolism—is somehow favored among all the chemical possibilities presented by the substances found in organisms. In order words, there must be information on which reactions to promote in living matter. This information is kept in the genes that ultimately encode the proteins that favor the reactions of life—enzymes. They do so by speeding up (i.e. catalyzing) these reactions, so that the substances participating in these reactions are much less available to react otherwise.

1 Overview of Metabolic Pathways

Metabolism is classically divided into catabolic and anabolic reactions. Anabolism comprises the reactions that lead to the formation of the complex and organized structures of living beings from smaller and simpler chemical building blocks. For anabolism to happen, there must be a continuous and substantial supply of chemical energy. This energy supply is derived from the reactions that make up the other branch of metabolism, that is catabolism. Catabolic reactions extract energy from chemical

F. Abdulkader (✉)
Department of Physiology and Biophysics, Instituto de Ciencias Biomedicas, Universidade de Sao Paulo, Sao Paulo, Brazil
e-mail: fkader@icb.usp.br

N. O. S. Camara et al. (eds.), *Essential Aspects of Immunometabolism in Health and Disease*, https://doi.org/10.1007/978-3-030-86684-6_2

substances by selectively and orderly breaking them up into simpler ones. In this process, the energy that kept their atoms together is partially but efficiently funneled to the synthesis of specific molecules, most important among these is adenosine triphosphate (ATP). In anabolism, ATP is used in tandem reactions that involve the transfer, and later removal, of phosphate from it to other molecules, yielding in the end ADP and inorganic phosphate. In this process, enzymes couple this use of ATP and the energy it releases with the synthesis of the complex biological molecules, and thus of the structures of life.

ATP synthesis, be it via substrate-level phosphorylation in the cytosol and mitochondrial matrix or oxidative phosphorylation in the mitochondrial inner membrane, is coupled to the oxidation of substrates, whose electrons are transferred to nicotinamide coenzymes, NADH or NADPH. NADH is an important source of electrons for the electron transport chain in mitochondria and thus of free energy for the chemiosmotic synthesis of ATP [2]. Meanwhile, NADPH is a source of reducing power for reductive processes in the cell, such as fatty acid synthesis and antioxidant defenses. Both NADH and NADPH are constantly recycled in redox reactions:

$$\mathrm{NAD(P)}^{+} + \mathrm{H}^{+} + 1\mathrm{e}^{-} \rightleftharpoons \mathrm{NAD(P)H}.$$

The molecules that are involved in metabolism can be broadly divided into four main classes: proteins, lipids, carbohydrates and nucleic acids. Each of these classes have chemical properties and biological functions that are characteristic of themselves, and each are formed in anabolism from specific small precursor molecules. Nucleic acids are made from nucleotides, which are composed of small nitrogenated bases, a five-carbon sugar and phosphate. The main function of nucleic acids in metabolism is indirect as bearers of genetic information, although they perform a crucial function for protein anabolism in ribosomes. Additionally, one of their nucleotide components is ATP, which also has the fundamental role in metabolism we have just discussed as energetic currency between catabolism and anabolism.

Despite sharing the feature of being also structural scaffolds in the living matter, proteins, carbohydrates and lipids have different functions in energy metabolism. Carbohydrates (sugars and their digestible polymers—starches and glycogen) mostly act as hydrophilic substrates for fast (anaerobic—cytosolic, oxygen-independent) or highly efficient (aerobic—mitochondrial, oxygen-dependent) ATP production. Carbohydrate metabolism can be considered the 'metabolic highway' of eukaryotic cells, as it shares many 'crossroads' with very important metabolic pathways involved with the other main metabolic substrates (Fig. 1). For instance, carbohydrates may be converted into lipids, which are an efficient form of chemical energy storage as, being hydrophobic, lipids (particularly triacylglycerols) do not carry along themselves solvating water molecules, thus reducing the space and weight associated with energy storage, were the organism to store energy only as carbohydrates. Lipids can be mobilized from their stores (mostly from white adipose tissue) in the form of free fatty acids, which also act as highly efficient substrates for ATP synthesis, though their catabolism is restricted to aerobic metabolism.

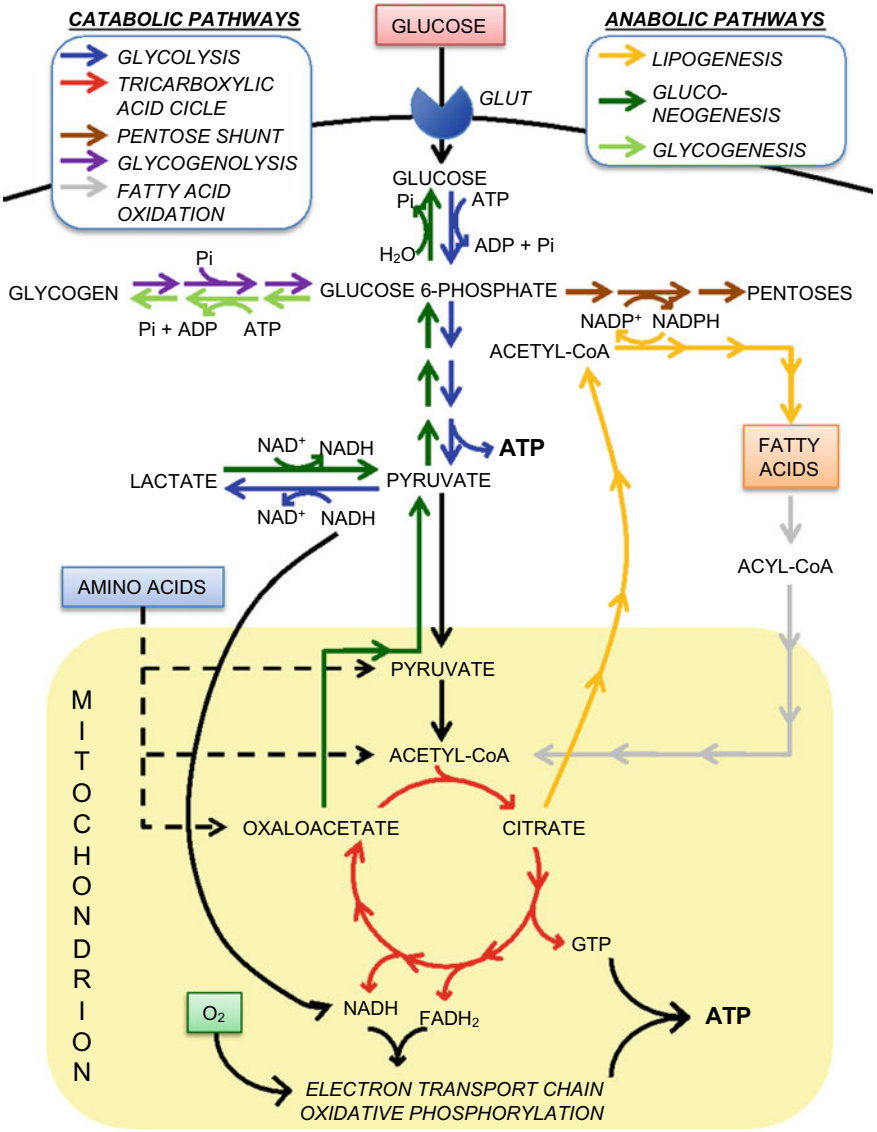


Fig. 1 An overview of the main enzymatic pathways involved in fuel metabolism. Both catabolic and anabolic pathways are sketched. The main substrates are highlighted in pink (glucose, the main carbohydrate), orange (fatty acids) and blue (amino acids)

As Fig. 1 illustrates, metabolic substrates are not freely interconvertible, and this is an issue particularly in the case of proteins. Proteins are not dedicated metabolic substrates, as they develop crucial functions in living organisms, as chemical (enzymes), mechanical (structural proteins), transport (channels and transporters) or communication (receptors) machines. However, their building blocks (amino acids) can yield carbohydrates and, therefore, lipids, via reactions that include the removal of their nitrogen atoms. So, between the three main metabolic substrates, only lipids cannot be converted into any of the others.

A brief analysis of Fig. 1 also demonstrates that metabolic pathways are extensively imbricated, and the final fate of substrates—or even which the substrates are—cannot be easily foreseen. However, despite the apparent complexity, metabolism is a highly dynamic but regulated process. An overview of the main instances of chemical signaling within metabolic regulation, as well as of nutrient and energy sensing by cells, is what lies ahead in this chapter.

2 The Mechanistic Logic of Metabolic Signaling

There are various levels and timeframes in which metabolic control can happen. The most basic type is the one that is intrinsic to a single metabolic pathway, in which the rate of catalysis of one or more enzymes is modulated by the reversible binding of intracellular molecules—usually end-products of the very same pathway—to specific non-catalytic sites of the enzyme. Upon binding, the conformation of the protein or enzyme is changed, either increasing or decreasing its affinity to the substrate(s) and/or its activity. This type of control of protein function, which is not restricted to enzymes, is known as allostery, and the molecules that mediate this phenomenon on a given protein are classified either as their positive or negative allosteric modulators depending on whether they increase or decrease its function [3, 4].

Within a metabolic pathway, allosteric modulation is the main mechanism in which feedback control happens [1]. For instance, in glycolysis, whose metabolic yield results in the net synthesis of 2 ATP molecules per glucose molecule metabolized, ATP is a negative allosteric modulator of the key enzyme phosphofructokinase 1 (PFK1). Conversely, AMP, which can be produced by the reaction of 2 ADP molecules to yield also one ATP by adenylate kinase in situations of ATP depletion, is a positive allosteric modulator of the same enzyme. However, allostery is in many cases also the framework for the integration of interconnected metabolic pathways. Here, again, PFK1 appears as an appropriate example. In situations in which metabolic flow through the tricarboxylic acid (TCA, or Krebs') cycle is much enhanced, such as in increased fatty acid oxidation, citrate can accumulate and allosterically inhibit PFK1, thus shifting ATP production from a carbohydrate to a lipid source [5]. This also stresses another common feature of metabolic control: usually the rate of catalysis of a pathway is determined by and controlled in a single or a few allosteric enzymes, which are effectively the master switches of that pathway.

Other proteins can also be the target of allosteric modulators that translate the metabolic state of the cell, with important signaling consequences within the same cell. For instance, there is a group of potassium ion channels that are allosterically modulated by the intracellular ATP/ADP concentration ratio. Aptly known as K_{ATP} channels, their open state probability is markedly decreased by raises in the [ATP]/[ADP] ratio, thus reducing potassium outflow and consequently causing membrane depolarization. This is the basis of the control of insulin secretion in pancreatic beta-cells by increased glucose availability and metabolism.

Metabolism-derived allosteric modulation of protein function is in this sense a universal feature of life as we know it. However, it is frequently taken for granted nowadays in many studies on metabolism, particularly in those focusing on multicellular organisms. This may be so because allostery could be seen as a phenomenon arising in single cells and affecting exclusively the same cells. However, the above example of K_{ATP} channel control of insulin secretion clearly demonstrates this is not always the case. In any case, allostery as it is commonly approached is a powerful and fast, but short-lived, mechanism of protein function modulation and thus of metabolic control, as it depends on the moment-by-moment levels of the allosteric modulators inside the cell.

Metabolic intercellular signaling is the domain of extracellular chemical mediators and nutrients. These molecules give rise to more perennial changes in the metabolic status of the cells upon which they act. The chemical basis of the sensing of either mediators or nutrients is, in itself, allosteric, as the proteins that first sense the signal transduce its level by changes in their own tertiary structure. However, the cascade of events downstream of this first sensing event tend to last much longer, as they imply in either covalent modifications of proteins (e.g. phosphorylation, dephosphorylation, prenylation etc.), changes in the concentration of intracellular second messengers (e.g. cyclic AMP, diacylglycerol etc.) [6], or modulation of the expression levels of specific proteins [1, 7]. This way, in metabolic intercellular signaling we are considering timeframes of several seconds to hours in principle, when considering covalent modifications and second messengers, to several minutes to days in expression modulation. However, some important intracellular metabolic sensing pathways utilize these same strategies, thus allowing individual cells to adapt to their individual energetic conditions and challenges.

Regarding covalent modifications of proteins in cell signaling, it is important to remember that the same protein may participate in distinct signaling pathways elicited by different extracellular mediators. As an example of that, consider phosphorylation. Phosphate groups from ATP can be incorporated into the structure of proteins as phosphate esters formed with sidechains of amino acids that bear a hydroxyl (OH) moiety, i.e. serine, threonine or tyrosine, in reactions catalyzed by protein kinase enzymes. There are two types of protein kinase enzymes regarding their amino acid substrate specificity: serine/threonine-kinases and tyrosine-kinases. So, a single signaling protein may be differentially modulated by phosphorylation depending on the kind of amino acid that is recognized by the protein kinase that phosphorylates it. Not only that, but within the same type of protein kinases considered, especially in the case of serine/threonine-kinases, a single protein may be phosphorylated in

different residues of the same amino acid, depending on the particular tertiary pattern each kinase recognizes.

Another often observed feature in post-translational modifications of proteins elicited by metabolic intercellular signaling is the fact that they may induce translocation of the targeted protein to different intracellular compartments. For instance, proteins of the signal transducer and activator of transcription (STAT) family dimerize and translocate from the cytoplasm to the nucleus upon specific tyrosine phosphorylation by Janus kinases (JAK). Once in the nucleus, they act as transcription factors and modulate protein expression. Hydrophobic modifications of proteins, such as prenylation or palmitoylation, are also important to localize/restrain signaling cascades to specific organelles or subcellular compartments as they tend to associate the modified proteins to specific membrane domains.

An important point that is often overlooked when discussing cell signaling in general is that, though the activated cascades last longer than the moment-by-moment allosteric modulation of enzyme activity, they do peter out. This has a clear adaptive value, as otherwise cells would be always inadvertently responding to signals and situations long gone. Various strategies of terminating signaling cascades in the absence of their stimulus exist, such as protein phosphatases, ubiquitin–proteasome degradation of activated proteins, degradation of second messengers (as is the case of phosphodiesterase-mediated hydrolysis of cyclic phosphates in cAMP and cGMP), slow intrinsic self-deactivating activity of signaling proteins (for instance the GTPase activity of the Gα subunits of G proteins) or the pumping out of calcium ions from the cytoplasm into either the endoplasmic reticulum (ER) or the extracellular space. Many of these strategies can be activated by the signaling cascade itself, generating negative feedback loops. Appropriately, there are even signaling pathways whose main effector proteins actually promote the deactivation of other signaling cascades, as happens in the inhibition of adenylate cyclase by $G\alpha_i$ proteins. This also sheds light into another outstanding feature of signaling cascades: they crosstalk, again because many signaling molecules and proteins are shared by them, and so the final observed effect is in every moment the result of the balance of their activity in these signaling hubs. As we will see, crosstalk is specially true for metabolic signaling [8–10].

Within this general logic of signaling strategies, in the next sections of this chapter we will briefly overview some examples of important pathways that mediate nutrient sensing in metazoans, as well as the signaling of some crucial hormones and mediators that affect whole-body metabolism. The following discussions do not intend by any means to be exhaustive, but merely to introduce the readers to some examples of the general mechanisms that were presented in this section in the hopes they will illuminate their comprehension of the many other pathways dealt with at depth in the other chapters of this book.

3 Nutrient-Sensing Signaling Pathways

As cell metabolism is basically dependent on ATP availability, which is coupled to NAD^+/NADH redox cycling, it is not surprising that signaling pathways that directly sense both variables have evolved.

In the case of ATP, this is brought about by a signaling system that is centered at a multiunit enzyme called 5'-AMP-dependent protein kinase, or AMPK [9, 11–16]. In situations of ATP depletion, the reaction catalyzed by adenylate kinase:

$$2\text{ADP} \rightleftharpoons \text{ATP} + 5'\text{AMP}$$

is shifted to the right, thereby increasing intracellular AMP content (note that this is not the cyclic form of AMP, cAMP, which is the second messenger produced upon activation of adenylate cyclase by $G\alpha_s$ proteins). AMP can bind to the regulatory γ subunit of AMPK and the consequent conformational change of the complex renders the catalytic α subunit more susceptible to phosphorylation, and thus activation, by liver kinase B1 (LKB1). Various proteins are substrates for active AMPK. This culminates in protein post-translational modifications and gene expression modulation that favor ATP production rather than ATP consuming processes. This way, among other actions, AMPK promotes lipolysis, fatty acid oxidation, glycolysis, glucose transport and autophagy, while inhibiting protein synthesis, lipogenesis, steroidogenesis and gluconeogenesis.

Nicotinamide coenzyme sensing, however, is mediated by another family of enzymes, sirtuins. These are lysine deacetylases that consume NAD^+ in their catalytic cycle, rather than reducing it to NADH as in its function of electron carrier in fuel metabolism. So, in situations in which substrate oxidation is reduced, the NAD^+/NADH ratio increases and consequently the availability of NAD^+ to sirtuins. There are seven sirtuins known in mammals (SIRT1-7), with different affinities for NAD^+, subcellular localization and substrate specificities [8, 16–18]. However, the ones that appear to have NAD^+-sensing abilities in the biological range of NAD^+ concentrations are the nuclear SIRT1 and the mitochondrial SIRT3 and SIRT 5. SIRT1 actions are the best described to date, and mainly involve the modulation of specific genes by deacetylation of specific transcription factors and cofactors, as well as histones. These culminate in the enhancement of mitochondrial function and overall glucose tolerance. Indeed, SIRT1 has been shown to increase the lifespan of animals subjected to high fat diets.

In eukaryotes, most of the ATP production is derived from aerobic mitochondrial metabolism. Consistently, oxygen availability also is sensed by cell signaling systems that impact on metabolism. This is mediated by a family of transcription factors known as hypoxia inducible factors (HIFs) [18–22]. Their function is controlled by an interesting mechanism in which the hydroxylation of HIF-1α, HIF-2α or HIF-3 α by prolyl hydroxylases that use O_2 as substrate signal for HIF proteasomal degradation at normal O_2 tensions. This way, in low O_2 levels, hydroxylation of α HIFs is reduced and the proteins accumulate, whereby they can migrate to the nucleus

and heterodimerize with constitutive HIF-1β and exert transcriptional control of hypoxia responsive element (HRE) genes. Some of these codify glycolytic enzymes and glucose transporters, thus increasing the cell capacity for anaerobic metabolism. On the other hand, it has been shown that HIF-1α in parallel reduces mitochondrial metabolism, both through effects on the conversion of pyruvate into acetyl-CoA and on the activity of TCA cycle enzymes, as well as on the biogenesis of mitochondria, through the indirect downregulation of a mitochondriogenesis-promoting transcriptional coactivator, PGC-1β. However, HIF signaling is not restricted to that, having pleiotropic effects on a multitude of processes important for tissue homeostasis, such as angiogenesis.

Mitochondrial metabolism also creates multiple signals that can be sensed by the cell. For instance, in addition to the ER, mitochondria are important intracellular calcium stores. Calcium mitochondrial content itself is an important regulator of oxidative phosphorylation [23]. However, at high levels and in the presence of oxidants, calcium can induce the formation in the inner mitochondrial membrane of a structure called permeability transition pore which leads to massive efflux of calcium to the cytosol and mitochondrial swelling to a point in which there is also release of cytochrome C. High cytosolic calcium and the presence of cytochrome C in the cytosol are known to trigger the intrinsic apoptotic pathway, for instance. But why would mitochondria be susceptible to oxidant damage? Incomplete reduction of oxygen in sites other than the final complex (IV) of the electron transport chain (ETC) seems to be the main source of superoxide anion, a reactive oxygen species (ROS) [9]. This gets more probable if there is a mismatch between the supply of electrons to the ETC from NADH and the rate of the ETC reactions, causing electrons to accumulate in the complexes of the ETC and increasing the chances they nonenzymatically react with molecular oxygen. This may happen either if the supply of oxygen to complex IV is low, or if the supply of NADH is higher than the capacity of the ETC to draw its electrons and pump protons, which can occur for instance if fatty acid oxidation (β-oxidation) is proceeding at very high rates. Reactive oxygen and nitrogen species are in themselves a feature of metabolism not only as otherwise unwanted byproducts, but also are controllers as well, as they trigger multiple signaling pathways which impact metabolism.

Apart from these mechanisms that we can consider to sense overall cellular energy status, we can also find in mammal cells an array of signaling pathways that specifically sense the three main groups fuel nutrients, the principles of which we will discuss in the next three subsections.

3.1 Lipid Sensing

Cells are endowed with pathways that sense the levels of fatty acids and that sense the availability of cholesterol, both kinds of signaling having major impacts on fuel metabolism.

Fatty acid sensing is mediated both by relatively fast G protein-coupled receptors (GPCRs) [6, 24, 25] and by slower, but longer-lasting, activation of intracellular receptors that are transcription factors, the peroxisome proliferator-activated receptors (PPARs) [26–29]. Fatty acid chemical properties are critical to their ability to activate the different subtypes of each family of fatty acid receptors. Among the GPCRs, GPR40, also known as free fatty acid receptor 1 (FFA1), is activated by medium to long chain fatty acids, particularly n-3 polyunsaturated fatty acids, while saturated fatty acids are more potent to activate it the longer their hydrocarbon chains. GPR40 is coupled to $G\alpha_q$ proteins and thus mediate its intracellular cascade via the activation of phospholipase C and thus the generation of the second messengers IP3, calcium (released from the endoplasmic reticulum) and diacylglycerol, which culminate in the activation of various enzymes, most notably C-type protein kinases (PKCs). More recently, another GPCR that is activated by long chain fatty acids has been described: GPR120 or free fatty acid receptor 4 (FFA4), whose intracellular signaling is similar to that for GPR40. However, in certain tissues GPR40 has been also shown to either stimulate or inhibit cAMP production by associating also to $G\alpha_s$ or $G\alpha_i$ proteins, respectively.

Meanwhile, short chain fatty acid levels, particularly acetate, propionate and butyrate, which are common byproducts of gut bacterial metabolism, are sensed by other GPCRs: GPR43, known also as free fatty acid receptor 2 (FFA2), and GPR41, alias free fatty acid receptor 3 (FFA3) [24]. While GPR43 signals via $G\alpha_i$ and $G\alpha_q$ proteins and GPR41 exclusively via $G\alpha_i$, it has been shown that GPR43 and GPR41 can heterodimerize. Interestingly, the heterodimer loses the ability to inhibit adenylate cyclase, while the raise in intracellular calcium levels is enhanced.

Long-term modulation of cell metabolism by fatty acids is mostly achieved by PPAR activation. Despite their name, PPARs do not restrict their actions only to peroxisomes, which are important organelles for the antioxidant defenses as well as for the metabolism of very long chain fatty acids in mammals and the production of fatty acid-derived signaling molecules. There are three isotypes of PPAR: PPARα, PPARγ and PPARδ (sometimes referred to as PPARβ/δ). Their distribution is not equal between tissues, the liver being the major site of expression of PPARα, while PPARγ is importantly expressed in adipose tissue and PPARδ has a more widespread distribution.

PPARα activation modulates gene expression in ways that enhance mitochondrial fatty acid oxidation not only in the liver, but also in muscle and adipose tissues. Moreover, fatty acid uptake is enhanced in the liver, but fatty acid export to the plasma in the form of lipoproteins is decreased, as well as cholesterol synthesis. This is the basis of the blood cholesterol-lowering effects of the PPARα agonists, fibrates. On the other hand, PPARγ activation (which is markedly promoted by n-3 fatty acids) is an important mediator of adipose tissue expansion and lipogenesis, while reducing leptin expression, and has an overall effect of increasing the whole-body sensitivity to insulin. This has been taken in advantage for the treatment of type 2 diabetes mellitus with the use of specific PPARγ agonists, the glitazones, as antidiabetic drugs. Also, while PPARγ increases liver output of LDL-cholesterol, it also increases apo-A secretion by the gut, thus increasing in parallel the capacity of

cholesterol scavenging by HDL. Both PPAR isotypes also have positive effects on pancreatic β cell function and, thus, on insulin secretion. Meanwhile, PPARδ is the least understood of the three kinds of PPAR so far, but PPARδ agonism has been shown to increase skeletal muscle utilization of glucose and fatty acid oxidation also in muscle, liver and in adipose tissue. Regardless of the PPAR isotype, the signaling involves translocation to the nucleus, where PPAR heterodimerizes with retinoic acid receptor (RXR) to modulate gene expression.

A distinct "flavor" to PPAR signaling is the family of proteins known as PPARγ coactivators (PGC) of transcription [8, 22, 30–32]. Now known not to be restricted as transcriptional coactivators only for PPARγ, PGCs are composed of PGC-1α (6 isoforms), PGC-1β and the PGC-1-related coactivator (PRC). Among them, PGC-1α is the most researched one and has been deemed the master regulator of mitochondriogenesis. In fact, PGC-1α can be considered a booster of whole-body oxygen-dependent oxidative metabolism, as it also promotes angiogenesis, muscle hypertrophy and liver gluconeogenesis.

As mentioned above, there are also sensing mechanisms for cholesterol found in mammalian cells. This is accomplished by a family of receptor transcriptional factors known as sterol regulatory element binding proteins, or SREBPs [1, 33]. Among them, we find two isoforms of SREBP-1, produced by alternative splicing of the SREBP-1 mRNA, and one of SREBP-2. The SREBP-1c isoform is mainly under the control of insulin and thus mediate the majority of the effects this hormone has on the expression of fatty acid metabolism genes in the liver and adipose tissue, mostly promoting lipogenesis. On the other hand, SREPB-2 signals for decreased cell cholesterol availability through an interesting mechanism, in which reduced cholesterol in the ER membrane renders it more available to migration to the Golgi and to proteolytic cleavage there. The N-terminal domain thus released can then migrate to the nucleus where it activates the gene expression of the LDL receptor and of enzymes of the cholesterol biosynthetic pathway.

3.2 Amino Acid Sensing

Though amino acids are known to stimulate both glucagon and insulin secretion, albeit to a much lesser extent than glucose influences the secretion of these metabolism-regulating hormones, a widespread system of cell signaling has evolved in eukaryotes that integrates many intracellular and extracellular signals, including amino acid availability (particularly the essential amino acid leucine in humans). This is centered on the serine/threonine kinase known as mechanistic target of rapamycin (formerly mammalian target of rapamycin), or mTOR [15, 34–36].

Depending on the proteins mTOR associates with, the signals received and the proteins it phosphorylates can be different. Thus, two mTOR complexes are recognized in mammalian cells: mTORC1 and mTORC2. mTORC1 is the complex for which most of the signaling has been described, and integrates various signals to promote anabolic effects, such as enhanced glucose catabolism and protein, lipid and

nucleotide synthesis, while inhibiting autophagy [37]. Overall, mTORC1 actions are an important drive for cell growth. Factors that stimulate mTORC1 activation are amino acids (but also other nutrients in general that increase overall energetic availability), hormones, oxygen and growth factors, while stress signals are inhibitory. On the other hand, mTORC2 seems to be mostly responsive to growth factors and promote cell survival and proliferation, mainly by enhancing glucose metabolism and mediating cytoskeletal rearrangements.

As noted above, the fate of mTOR between these two kinds of complexes is determined by the proteins to which it is associated. In mTORC1, mTOR is bound to RAPTOR, a protein that integrates various upstream signals, as well as to FKBP12, which is the protein when bound to rapamycin inhibits mTORC1 function. In mTORC2, RICTOR plays the role that RAPTOR has of signal integration in mTORC1. Interestingly, rapamycin only decreases mTORC2 activity in chronic treatments.

3.3 Carbohydrate Sensing

Regulation of carbohydrate metabolism is mainly brought about by whole-body coordination of functions through the pancreatic islet hormones, insulin and glucagon. However, it has been shown that in cell preparations lacking either these hormones, glucose independently influences the expression of genes that promote fat storage through a pathway that is mediated by a transcription factor known as carbohydrate responsive element binding protein, or ChREBP [1]. With increased glucose availability, metabolic flux through the pentose pathway also increases, and one of its products, xylulose 5-phosphate, can now activate a protein phosphatase, PP2A, that dephosphorylates ChREBP. This way, ChREBP can then migrate to the nucleus and activate lipogenesis genes, thus favoring the conversion of the excess carbohydrate into fatty acid stores.

4 Extracellular Signals that Regulate Fuel Metabolism

In pluricellular organisms, the concerted control of fuel metabolism in different tissues and organs certainly gave them an adaptive advantage to cope with changes in the environment that would imply either into substrate bounty or scarcity. In vertebrates, and particularly mammals, a sophisticated control system with multiple feedback loops and redundancy evolved that allows them to regulate the extracellular availability of metabolic substrates, particularly glucose, within narrow ranges of concentration. This control system integrates both the nervous and the endocrine systems, and acts via changes in feeding behavior, but most of all by changing the flux of metabolic substrates (sugars, lipids and amino acids) to and from organs

(mainly the liver, skeletal muscle and white adipose tissue) that can produce or store these substrates for their own use or for the disposal of the whole body.

But why glucose, of all the metabolic substrates, was selected by evolution to be the main surrogate for overall nutrient availability in this complex control system? For one side, amino acids are not properly storable, as their polymers are proteins, with their own individual and important functions. In addition, and crucially, there are tissues that are exclusively glycolytic, such as red blood cells, that in this way cannot depend on the mitochondrial oxidation of other substrates for ATP production. (Incidentally, in the case of erythrocytes that would be clearly an adaptive nonsense as they would consume for their own good the main substance their job is to transport across the whole body, oxygen). Moreover, the nervous tissue is in normal situations strictly dependent on glucose metabolism to supply its energetic demand. And this demand can be huge: a simple back of the envelope calculation demonstrates that the amount of glucose available in blood at any time (100 mg/dL in roughly 5 L of blood, so 5 g of glucose) would only suffice to support brain function for just one hour in the unreal situation in which the brain would be the only tissue using that glucose (the mass of glucose daily used by the central nervous system amounts to 120 g, something like a handful of sugar). This testifies to the fact that the flux of glucose to and from the blood is intense and tightly matched. Of course, other nutrients are also sensed by the nervous and endocrine system, such as bodily fat content via the proportional secretion of leptin by the adipose tissue, which signals to the hypothalamus to inhibit feeding behavior and to increase the sympathetic drive to adipose territories where it promotes lipolysis and fatty acid oxidation. Also, amino acids do stimulate insulin and glucagon secretions, albeit to a much smaller extent than the effects brought about on them by changes in blood glucose concentration.

This way, the extracellular signaling landscape can change dramatically and many times during a single day, depending on how many times we eat, what we eat, and how much. At the obvious risk of oversimplification, we can for didactic purposes consider a regular day in the life of a human from the standpoint of glucose metabolism as the alternation of periods when there is an external input of glucose through feeding, and periods when we are fasting, in which this external glucose input is no more.

In the fed state, blood glucose concentration tends to rise, and this increased glucose availability results in increased glucose uptake, metabolism and ATP concentration in pancreatic β cells. ATP closes K_{ATP} channels in these cells, which depolarizes them, leading to the opening of voltage-gated calcium channels and thus to the increased intracellular calcium concentration that triggers the exocytosis of preformed granules of insulin. This glucose sensing mechanism is clearly very fast, producing raises in blood insulin concentration quickly and in phase with the increases in blood glucose. Insulin signaling via its receptor is very fast too and, though it can be found in many tissues, making insulin a general growth factor, the main whole-body effects of this hormone on metabolism are due to its effects on the liver, adipose tissue and skeletal muscle, as illustrated in Fig. 2. Basically, insulin can be regarded as an anabolic hormone that promotes glycogen, protein and fat (triacylglycerol, TAG) synthesis by increasing glucose uptake and/or use in these organs as well as inhibiting glucose production in the liver and free fatty acid

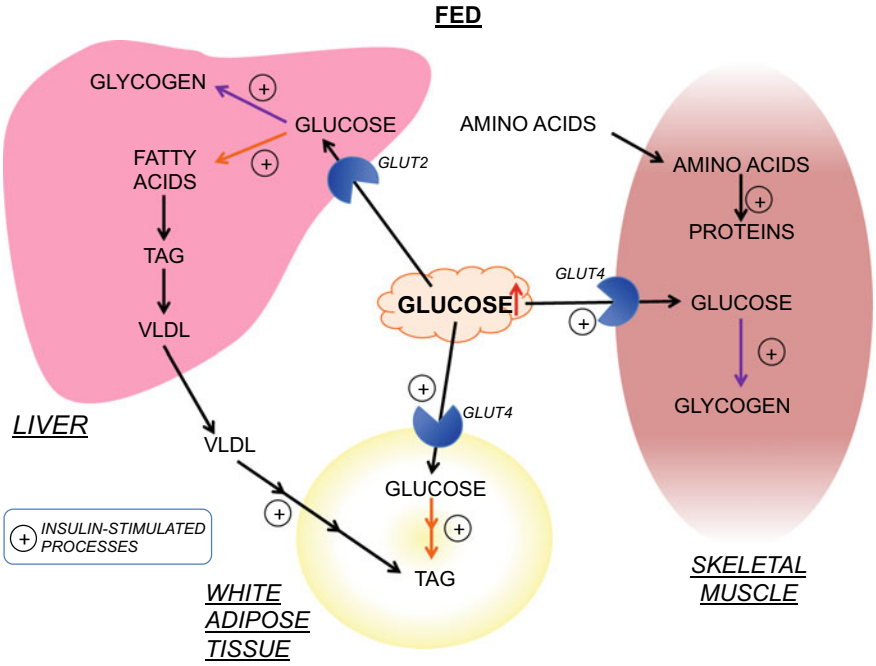


Fig. 2 Interplay between the liver, skeletal muscle and white adipose tissue in the fed state. The colors of the pathways depicted correspond to the same pathways in Fig. 1

output. Using the biochemical jargon, insulin stimulates glycolysis and glycogenesis in liver and muscle, glucose transport in the muscle and adipose tissue, protein synthesis mainly in muscle (in part by the increased intracellular availability and metabolism of glucose), lipogenesis from glucose in liver and adipose tissue, and inhibits glycogenolysis and gluconeogenesis in the liver, and lipolysis in the adipose tissue.

How does insulin mediate all these effects? The insulin receptor is a tyrosine protein kinase that is activated upon insulin binding. Not only does it phosphorylate tyrosine residues in specific substrates (insulin receptor substrate 1, IRS-1, and insulin receptor substrate 2, IRS-2), but it also phosphorylates itself. The phosphotyrosine motifs thus generated are recognized and bound to by proteins that bear the Src homology domain 2 (SH2). The IRS also have this domain and get recruited to the site of the insulin receptor by its autophosphorylation, as well as other proteins containing SH2, such as the p85 regulatory subunit of phosphoinositol-3-kinase (PI3K). This brings the catalytic p110 subunit of PI3K into close vicinity to the plasma membrane, where it can access its substrate, phosphatidylinositol-4,5-bisphosphate, and phosphorylate carbon 3 in the inositol moiety, producing phosphatidylinositol-3,4,5-triphosphate. This is recognized by proteins that have a domain called pleckstrin homology domain, such as the phosphoinositide-dependent kinase 1 (PDK-1). This protein thus activated phosphorylates a most important downstream protein in this

cascade, protein kinase B (PKB), more commonly known as Akt, on its threonine 308. This renders Akt partially active, what is fully achieved by concurrent phosphorylation at serine 473 by mTORC2. Akt itself is a serine/threonine protein kinase and has various possible substrates, including mTORC1 and glycogen synthase kinase 3 (GSK-3). While phosphorylation by Akt activates mTORC1, it inhibits GSK-3 activity. Despite its name, GSK-3 action is not restricted to glycogen synthase, whose phosphorylation by GSK-3 inhibits its function. Incidentally, this release from inhibition of glycogen synthase from GSK-3-mediated phosphorylation by Akt is the mechanism by which insulin induces glycogen synthesis. In addition to these examples of fast modulation of cell metabolism by insulin through Akt, insulin and Akt also have effects on gene expression, such as the aforementioned effect on SREBP-1c.

As insulin actions ensue, blood glucose levels accordingly reduce, ceasing to be a stimulus for further insulin secretion. The reduction of insulin levels itself already brings the insulin-responsive tissues to a more catabolic state. However, falling glucose levels during fasting are also detected by both the central nervous system and the pancreatic α cells that culminate in the release of catabolic mediators that increase glucose levels, namely noradrenaline, adrenaline and glucagon. Other hormones exist that also raise glycemia, and their secretion can also be enhanced by reductions in blood glucose as a stress factor, such as glucocorticoids, growth hormone and the thyroid hormones. This shows that insulin faces rather unfair competition as the single glucose-lowering hormone in humans.

Both glucagon and (nor)adrenaline signal to the metabolism-controlling organs via G protein-coupled receptors associated with $G\alpha_s$ subunits that activate adenylate cyclase to produce cAMP. Increased cAMP activates the serine/threonine protein kinase A (PKA) and, in higher concentrations, also another signaling pathway whose importance has been more recently acknowledged, mediated by cAMP-regulated guanine nucleotide exchange factors (cAMP-GEFs), also known as exchange factors directly activated by cAMP, or Epac. Though the glucagon receptor is found in other tissues, in humans its action is basically restricted to the liver, as it is secreted into the portal bed that drains into the liver, where most of the glucagon secreted is metabolized, making its systemic concentration marginally effective in adipose tissue and muscle.

Cyclic AMP signaling promotes effects that counteract those of insulin in the liver (Fig. 3). For instance, PKA activation stimulates glycogenolysis (via phosphorylation of phosphorylase kinase, which activates glycogen phosphorylase) and inhibits glycogen synthesis (through glycogen synthase phosphorylation, akin to that promoted by GSK-3). Also, gluconeogenesis is enhanced, through the increased availability of amino acids and lactate from muscle protein and glycogen catabolism, respectively. However, direct adrenaline action and cAMP signaling in muscle promotes protein synthesis rather than degradation. Another important substrate for gluconeogenesis is glycerol derived from adipose tissue lipolysis (which is also stimulated by cAMP through (nor)adrenaline). The concurrent increased free fatty acid output from lipolysis in white adipose tissue would lead to increased fatty acid oxidation in the liver, as it happens in other tissues which are thus offered an alternative to glucose as metabolic substrate, thus helping to reduce glucose consumption and thus

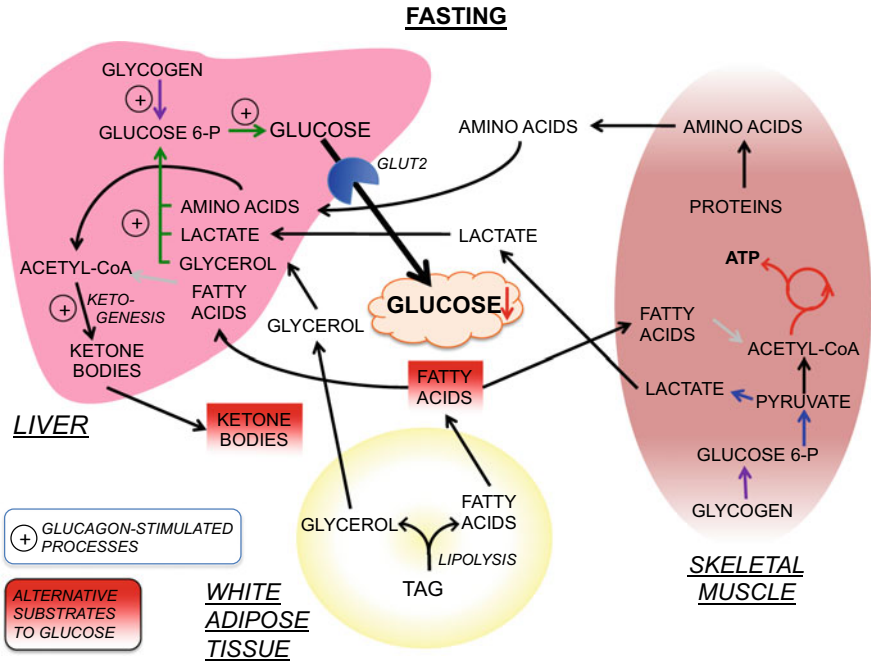


Fig. 3 Interplay between the liver, skeletal muscle and white adipose tissue in the fasting state. The colors of the pathways depicted here correspond to the same pathways in Fig. 1

to maintain blood glucose levels. However, as gluconeogenesis is stimulated in the liver, which uses TCA cycle intermediates as substrates, the activity of the TCA cycle is reduced, and acetyl-CoA builds up. The accumulated acetyl-CoA can be condensed into acetoacetate and β-hydroxy-butirate, known as ketone bodies. In prolonged fasting, as β-hydroxy-butirate concentration elevates, this ketone body induces the expression of its own metabolizing enzyme, β-hydroxy-butirate dehydrogenase, in the brain, which adapts this way its metabolism to use ketone bodies as alternative substrates to glucose. However, the increased ketogenesis leads to metabolic acidosis, which in part is compensated by the induction of proton-consuming gluconeogenesis in the kidneys, as the gluconeogenic enzymes there are induced by a curious mechanism in which the stability of their mRNA increases in low pH.

By this finely concerted interplay of tissues through metabolic signaling, humans are able to endure many weeks in fast, giving testimony to the selective pressures that prevailed in our dire evolutionary history of scarcity, rather than plenty. It is only very recently that plenty has become the norm, rather than the still sad exception, in most human societies. And so, as a species we have not had time to adapt to this excess food availability, thus explaining the sharp increase in metabolic and obesity-related diseases that will be considered in other chapters of this book [38, 39].

5 Pathway Crosstalk in the Regulation of Metabolism

As we have seen so far, cell metabolism is both subject to intrinsic signals in each cell itself that are derived from its own nutrient supply and to extracellular chemical mediators that integrate and signal the overall metabolic state of the organism. As expected, both these levels in signaling interact, as they may share common signaling proteins, into what is known as pathway crosstalk. Figure 4 is a crude attempt to show a very incomplete account of the possibilities of crosstalk in some of the pathways we have discussed in this chapter. The complexity of this network is evident, which could be seen maybe as an impediment to comprehension and research in this field. However, it is also a display of the robustness and sensitivity of metabolic signaling, a clear evidence of the evolutionary advance and sheer reliability it offers. With the outstanding advance in computational power, as well as the growing interdisciplinary interest that metabolism draws, systems biology approaches are steadfastly increasing our comprehension of these interactive levels of control and complexity [9, 10].

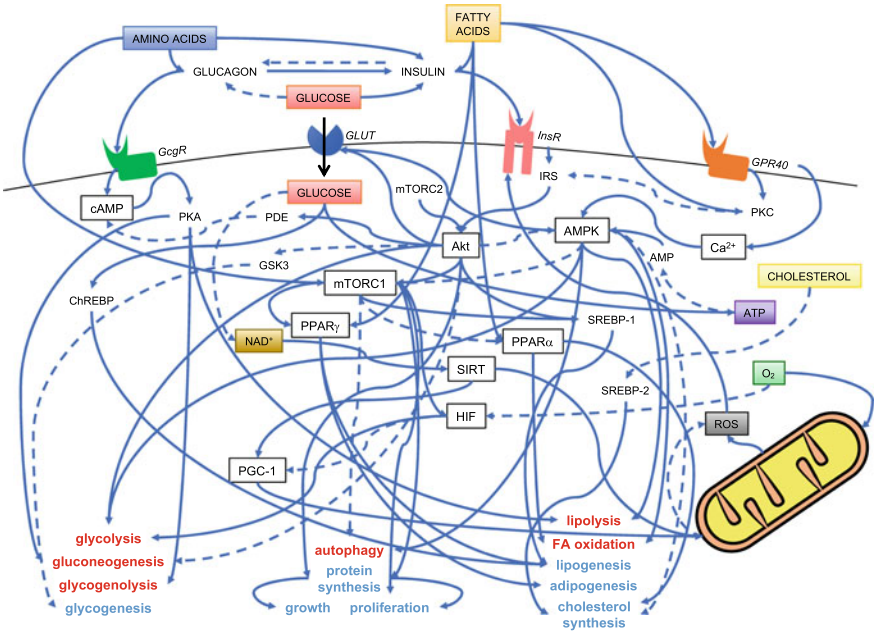


Fig. 4 Crosstalk between pathways in metabolic signaling. Nutrients and other signals that are sensed appear in colored boxes. The main signaling hubs are depicted in white boxes. Anabolic processes are written in blue boldface whereas catabolic processes are in red boldface. Solid arrows depict stimulatory interactions, whereas broken arrows represent inhibitory interactions

References

1. Frayn KN (2010) Metabolic regulation: a human perspective, 3rd edn. Wiley-Blackwell, Oxford
2. Cantó C, Menzies KJ, Auwerx J (2015) NAD^+ Metabolism and the control of energy homeostasis: a balancing act between mitochondria and the nucleus. Cell Metab 22:31–53
3. Monod J, Changeux J-P, Jacob F (1963) Allosteric proteins and cellular control systems. J Mol Biol 6:306–329
4. Monod J, Wyman J, Changeux J-P (1965) On the nature of allosteric transitions: a plausible model. J Mol Biol 12:88–118
5. Randle PJ, Garland PB, Hales CN, Newsholme EA (1963) The glucose fatty-acid cycle. Its role in insulin sensitivity and the metabolic disturbances of diabetes mellitus. Lancet 281(7285):785–789
6. Husted AS, Trauelsen M, Rudenko O, Hjorth SA, Schwartz TW (2017) GPCR-mediated signaling of metabolites. Cell Metab 25:777–796
7. Haro D, Marrero PF, Relat J (2019) Nutritional regulation of gene expression: carbohydrate-, fat- and amino acid-dependent modulation of transcriptional activity. Int J Molec Sci 20:1386
8. Sugden MC, Caton PW, Holness MJ (2010) PPAR control: it's SIRTainly as easy as PGC. J Endocrinol 204:93–104
9. Thirupathi A, Souza CT (2017) Multi-regulatory network of ROS: the interconnection of ROS, PGC-1 alpha, and AMPK-SIRT1 during exercise. J Physiol Biochem 73:487–494
10. Watson E, Yilmaz LS, Walhout AJM (2015) Understanding metabolic regulation at a systems level: metabolite sensing, mathematical predictions, and model organisms. Annu Rev Genet 49:553–575
11. Burkewitz K, Zhang Y, Mair WB (2014) AMPK at the nexus of energetics and aging. Cell Metab 20:10–25
12. Carling D, Viollet B (2015) Beyond energy homeostasis: the expanding role of AMP-activated protein kinase in regulating metabolism. Cell Metab 21:799–804
13. Hardie DG (2014) AMP-activated protein kinase: a key regulator of energy balance with many roles in human disease. J Intern Med 276:543–559
14. Hardie DG (2014) AMPK-sensing energy while talking to other signaling pathways. Cell Metab 20:939–951
15. Hindupur SK, González A, Hall MN (2015) The opposing actions of target of rapamycin and AMP-activated protein kinase in cell growth control. Cold Spring Harb Perspect Biol 7:a019141
16. Boutant M, Canto C (2014) SIRT1 metabolic actions: integrating recent advances from mouse models. Mol Metab 3:5–18
17. Lin S-C, Hardie DH (2018) AMPK: sensing glucose as well as cellular energy status. Cell Metab 27:299–313
18. Schwer B, Verdin E (2008) Conserved metabolic regulatory functions of sirtuins. Cell Metab 7:104–112
19. Yu Q, Dong L, Li Y, Liu G (2018) SIRT1 and HIF1a signaling in metabolism and immune responses. Cancer Lett 418:20e26
20. Choudhry H, Harris AL (2018) Advances in hypoxia-inducible factor biology. Cell Metab 27:281–298
21. Metzen E, Ratcliffe PJ (2004) HIF hydroxylation and cellular oxygen sensing. Biol Chem 385:223–230
22. Semenza GL (2004) Hydroxylation of HIF-1: oxygen sensing at the molecular level. Physiology 19:176–182
23. Shoag J, Arany Z (2010) Regulation of hypoxia-inducible genes by PGC-1α. Arterioscler Thromb Vasc Biol 30:662–666
24. Vercesi AE, Castilho RF, Kowaltowski AJ, Oliveira HCF, Souza-Pinto NC, Figueira TR, Busanello ENB (2018) Mitochondrial calcium transport and the redox nature of the calcium-induced membrane permeability transition. Free Radic Biol Med 129:1–24
25. Ang Z, Xiong D, Wu M, Ding JL (2018) FFAR2-FFAR3 receptor heteromerization modulates short-chain fatty acid sensing. FASEB J 32:289–303

26. Kimura I, Ichimura A, Ohue-Kitano R, Igarashi M (2020) Free fatty acid receptors in health and disease. Physiol Rev 100:171–210
27. Charo IF (2007) Macrophage polarization and insulin resistance: PPARg in control. Cell Metab 6:96–98
28. Dubois V, Eeckhoute J, Lefebvre P, Staels B (2017) Distinct but complementary contributions of PPAR isotypes to energy homeostasis. J Clin Invest 127(4):1202–1214
29. Hong F, Pan S, Guo Y, Xu P, Zhai Y (2019) PPARs as nuclear receptors for nutrient and energy metabolism. Molecules 24:2545
30. Zechner R, Zimmermann R, Eichmann TO, Kohlwein SD, Haemmerle G, Lass A, Madeo F (2012) FAT SIGNALS—lipases and lipolysis in lipid metabolism and signaling. Cell Metab 15:279–291
31. Besseiche A, Riveline J-P, Gautier J-F, Bréant B, Blondeau B (2015) Metabolic roles of PGC-1α and its implications for type 2 diabetes. Diabetes Metab 41(5):347–357
32. Martínez-Redondo V, Petterson AT, Ruas JL (2015) The hitchhiker's guide to PGC-1α isoform structure and biological functions. Diabetologia 58:1969–1977
33. Supruniuk E, Mikłosz A, Chabowski A (2017) The implication of PGC-1a on fatty acid transport across plasma and mitochondrial membranes in the insulin sensitive tissues. Front Physiol 8:923
34. Shao W, Espenshade P (2012) Expanding roles for SREBP in metabolism. Cell Metab 16:414–419
35. Lamming DW, Sabatini DM (2013) A central role for mTOR in lipid homeostasis. Cell Metab 18:465–469
36. Liu GY, Sabatini DM (2020) mTOR at the nexus of nutrition, growth, ageing and disease. Nat Rev Mol Cell Biol 21(4):183–203
37. Singh R, Cuervo AM (2011) Autophagy in the cellular energetic balance. Cell Metab. 13:495–504
38. Baker RG, Hayden MS, Ghosh S (2011) NF-κB, inflammation, and metabolic disease. Cell Metab 13:11–22
39. Vitale I, Manic G, Coussens LM, Kroemer G, Galluzzi L (2019) Macrophages and metabolism in the tumor microenvironment. Cell Metab 30:36–50

Mitochondrial Biogenesis and Dynamics in Health and Disease

Juliane C. Campos, Luiz H. M. Bozi, and Julio C. B. Ferreira

1 Introduction

Mitochondria are double-membrane-bound organelles exclusively found in eukaryotic cells and best known for its role in the generation of adenosine triphosphate (ATP) [1]. The endosymbiotic hypothesis proposes that mitochondria arise from the integration of a free-living aerobic bacterium into a host cell over a billion of years ago. In this relationship, the host cell provided a safe and nutrient-rich environment for the aerobic bacterium. It also acquired a new source of oxygen dependent-energy [2]. More recently, it has been suggested that the phagocytosed bacterium may have provided defense molecules for the host cell, also connecting the advantages of this endosymbiotic relationship to immunity [3]. Throughout evolution, a massive transfer of genes to the host cell allowed the evolvement of the endosymbiotic bacterium as a permanent organelle—the mitochondrion (mitochondria for plural) [4].

J. C. Campos · L. H. M. Bozi · J. C. B. Ferreira (✉)
Institute of Biomedical Sciences, University of Sao Paulo, Sao Paulo, Brazil
e-mail: jcesarbf@usp.br

J. C. Campos
e-mail: jucampos@usp.br

L. H. M. Bozi
e-mail: luizbozi@usp.br

J. C. Campos
Joslin Diabetes Center and Department of Genetics, Harvard Medical School, Boston, USA

L. H. M. Bozi
Dana-Faber Cancer Institute and Department of Cell Biology, Harvard Medical School, Boston, USA

J. C. B. Ferreira
Department of Chemical and Systems Biology, School of Medicine, Stanford University, Stanford, USA

N. O. S. Camara et al. (eds.), *Essential Aspects of Immunometabolism in Health and Disease*, https://doi.org/10.1007/978-3-030-86684-6_3

Derived from two Greek words: "*mitos*"—thread and "*chondros*"—granule, the organelle displays two lipid bilayer-membranes enclosing two aqueous compartments. The outer mitochondrial membrane surrounds the intermembrane space, while the inner mitochondrial membrane, which contains invaginations denominated cristae, encloses the matrix compartment [5, 6]. The inner membrane accommodates the oxidative phosphorylation system (OXPHOS)—a five multimeric protein complexes (Complex I–V) that uses redox reactions to generates ATP. At the expense of oxygen as a final electron acceptor, a sequential transfer of electrons from Complex I to Complex IV generates a proton electrochemical gradient across the inner membrane, also known as membrane potential, that is used by Complex V to drive ATP synthesis [1]. The mitochondrial membrane potential also helps metabolites transport and ion homeostasis.

Often referred as the powerhouse of the eukaryotic cells, the role of mitochondria goes beyond ATP production. The organelle orchestrates a myriad of other processes including reactive oxygen species (ROS) formation [7, 8], aldehyde metabolism [9], heat production [10], ion homeostasis [11] and programmed cell death [12] that ultimately dictate cell fate. This functional versatility is intimately linked to the content, size and number of mitochondria. Their morphological complexity is controlled by the processes termed mitochondrial biogenesis and dynamics. While mitochondrial biogenesis increases the number and content of the organelles in a coordinated effort with the nucleus [13], mitochondrial dynamics drives the formation of larger or smaller organelles through the antagonist activities of fusion and fission [14].

Mitochondria are recently recognized by their dynamic nature. In order to meet the cellular requirements for ATP, the mitochondrial network are under constantly remodeling. Indeed, metabolic cues (i.e. starvation, exercise) trigger not only fusion and fission machineries in order to create elongated or fragmented mitochondria [15], but also drive transcription factors activation to increase mitochondrial mass and boost oxidative metabolism [16]. Moreover, this dynamism helps impaired mitochondria to be rescued or eliminated. In the first case, fusion events allow damaged components to be diluted throughout the network, thereby avoiding the propagation of stress that might cause mitochondrial dysfunction or collapse [17]. On the opposite way, the fission process segregates part of dysfunctional mitochondria that now can be addressed for degradation in the lysosome, a process termed mitophagy [18].

Exciting new findings have revealed mitochondria as a major intracellular signaling platform regulating immune cell function. Indeed, the cellular metabolic plasticity provided by mitochondria not only allows immune cells to grow, but it is also required during transition from a metabolically quiescent stage to a highly active state [19]. Moreover, proteins located on the outer mitochondrial membrane, as well as mitochondrial DNA can dictate immune cell activation [20, 21]. Finally, due to the reciprocal crosstalk between mitochondrial metabolism and morphology, fluctuations in shape, size and position of the organelle within the cell likey affect both phenotype and activity of immune cells [22].

Considering the extensive knowledge highlighting mitochondria as the powerhouse of the cells as well as emerging evidence placing mitochondria at the heart of immunity, this chapter reviews the general processes regulating mitochondrial

biogenesis and dynamics, and discuss the critical role of these processes in health and disease.

2 Mitochondrial Biogenesis

Mitochondrial biogenesis is a simplified term used to describe a complex process involving the increase in mass of pre-existing mitochondria. Due to their bacterial origin, mitochondria possess their own genetic material, which includes DNA and the translational/transcriptional system. The mitochondrial DNA (mtDNA) is a circular double strand DNA molecule containing ~16.5 kb that encodes only 37 genes: 22 transfer RNA and 2 ribosomal RNA (12S and 16S) required for translating 13 messenger RNA. Moreover, maternal inheritance, lack of introns (non-coding sections of a gene) and several copies per cell (1–10 copies per mitochondrion) are among the unique features that differ mtDNA from the nuclear DNA [23].

The entire protein-coding capacity of mtDNA relies on 13 essential subunits of the electron transport chain (ETC) that are replicated and transcribed within the mitochondrial matrix: 7 subunits of NADH: Ubiquinone oxidoreductase (Complex I), 1 subunit of Ubiquinone: Cytochrome c oxidoreductase (Complex III), 3 subunits of Cytochrome c Oxidase (Complex IV) and 2 subunits of ATP synthase (Complex V) [24]. The ~1100 remaining mitochondrial proteins [25, 26] have to be transcribed in the nucleus, translated in cytosolic ribosomes and imported into the organelle (Fig. 1). Therefore, mitochondrial biogenesis faces several challenges before promoting an increase in the mitochondrial content.

The first challenge relies on coordinating the gene expression between two genomes located into distinct subcellular compartments. Indeed, to ensure a proper OXPHOS, the number of ETC subunits must be stoichiometrically balanced [27]. mtDNA occurs in the ratio of ~1000:1 copies relative to nuclear DNA [23]. Second, the majority of mitochondrial proteins are translated in the cytosol; thus, demanding a synchronized cellular machinery to properly target, import and assemble these nuclear-encoded proteins [28, 29]. Failure in addressing these proteins to mitochondria not only impairs ETC subunitse stoichiometry, but also compromises mtDNA replication, which is orchestrated by the nuclear-encoded protein DNA polymerase *gamma* (POLG) [30]. For a complete description about how mitochondrial genome is replicated, transcribed and translated, please see reviews [28, 31, 32]. Finally, mitochondrial dynamics, which will be discussed above, must also be coordinated.

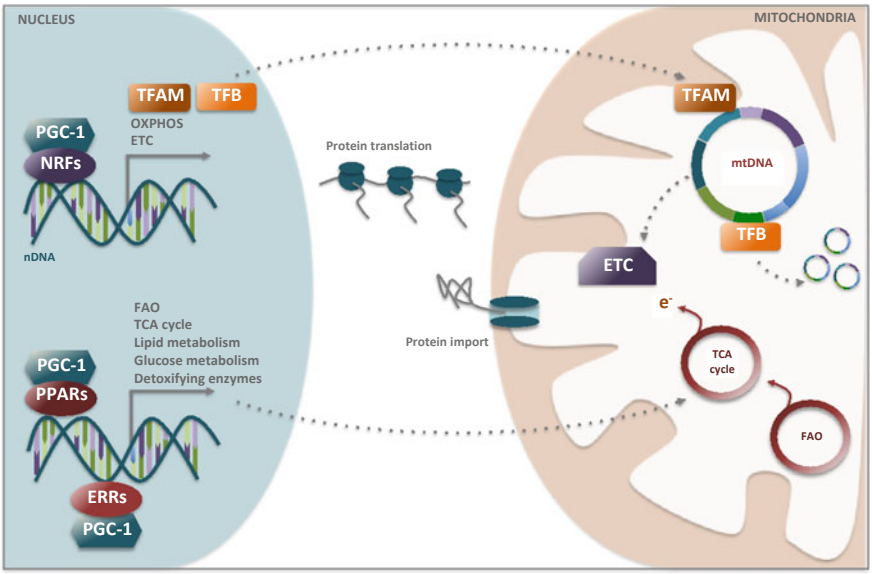


Fig. 1 Summary of the transcriptional regulation of mitochondrial biogenesis. The expression of mitochondrial genes encoded by both nDNA (nuclear DNA) and mtDNA (mitochondrial DNA) is mainly regulated by a family of transcriptional coactivators named PGC-1 [peroxisome proliferator-activated receptor (PPAR) gamma coactivator 1]. PGC-1 members bind to and coactivate NRFs (nuclear respiratory factors) to induce the expression of multiple components of the OXPHOS (oxidative phosphorylation system), ETC (electron transport chain) and mtDNA replication. NRFs also regulate the levels of TFAM and TFB (mitochondrial transcription factors A and B, respectively) involved in the expression of genes encoded by the mtDNA. Interaction between PGC-1 and specific transcription factors such as PPARs and EERs (estrogen-related receptors) control the expression of many genes involved in FAO (fatty acid oxidation), TCA cycle (tricarboxylic acid cycle), glucose and lipid metabolism, and detoxifying enzymes. Nuclear-encoded mitochondrial proteins are translated in cytosolic ribosomes and imported into the organelle

2.1 Transcription Factors Regulating Mitochondrial Biogenesis

The transcription of both nuclear and mitochondrial genomes is coordinated by specific proteins termed transcription factors. Transcription factors are able to modulate the rate of gene expression by binding to specific regulatory regions of DNA. These proteins contain effector domains that allow the interaction not only with other proteins essential for transcription, including the RNA polymerase, but also with other transcription factors; thereby regulating the amount of messenger RNA produced per gene [33].

Nuclear Respiratory Factors 1 (NRF-1) and 2 (NRF-2) are considered critical players in mitochondrial biogenesis. Together, these transcription factors display

DNA-binding sites for most of the genes encoding respiratory subunits. First identified in 1989 as a transcriptional activator of the cytochrome c gene [34], NRF-1 targeted genes are now branded for encoding subunits of all five respiratory complexes of the ETC [35]. A couple of years later, NRF-2 was discovered by its specific binding to the cytochrome oxidase subunit IV promoter [36]. Although often recognized by their power of binding to antioxidant response element (ARE) and promoting gene expression of detoxifying enzymes [37], functional NRF-2 sites have been implicated in the expression of subunits of Complex II, IV and V of the OXPHOS [38].

The regulatory network of NRFs also targets other nuclear genes whose products function in the mitochondria, including components for assembling and importing mitochondrial proteins [39], and constituents of the mtDNA transcription and replication machinery [40]. Indeed, NRF-1 is able to stimulate the expression of mitochondrial transcription factors A (TFAM) [40] and B (TFB) [41]—two nuclear-encoded transcription factors essential for replication, maintenance, and transcription of mtDNA [42, 43]. Moreover, not only TFAM and TFB have been recently added to the list of genes controlled by NRF-2 [38], but NRF-2 indirectly regulates mitochondrial biogenesis by driving the gene expression of NRF-1 [44]. Due to this essential role in coordinating bi-genomic respiratory subunits, deficiency of NRF can lead to a severe impairment of mitochondrial biogenesis [45, 46].

Members of the nuclear receptor superfamily also control the transcription of respiratory apparatus. The peroxisome proliferator-activated receptor (PPAR) family is composed by three isoforms: PPARα [47], PPAR β/δ [48], PPARγ [49]. The expression of PPAR isoforms differs among tissues and these transcription factors regulate metabolic pathways at different levels. While PPARγ is involved in glucose metabolism and regulation of fatty acid storage, PPARα and PPAR β/δ promote changes in cellular lipid metabolism by upregulating genes involved in mitochondrial fatty acid oxidation [50]. Estrogen-related receptors α (ERRα) and γ (ERRγ) represent another class of nuclear receptors targeting ~700 nuclear-encoded mitochondrial genes. The controlling of these transcription factors, expressed in mitochondrion-enriched tissues such as skeletal muscle and heart [51], is attached to all aspects of energy homeostasis, including mtDNA replication, OXPHOS, ion homeostasis and mitochondrial detoxifying mechanisms (reviewed in [52]). Moreover, ERRα can regulate the levels of PPARα transcripts [53], therefore magnifying the control over mitochondrial fatty acid oxidation pathway.

Finally, a relative small number of other transcription factors have been shown to activate or repress nuclear genes encoding mitochondrial proteins, including stimulatory protein 1 (Sp1), ying yang 1 transcription factor (YY1), cAMP-responsive element-binding protein (CREB) and myocyte enhancer factor 2 (MEF-2), and a detailed consideration of those is covered elsewhere [54].

2.2 The Role of Transcriptional Coactivators in Mitochondrial Biogenesis: PGC-1 Family

As described above, mitochondrial biogenesis requires the coordination of several transcription factors to proper ensure the expression of both nuclear and mitochondrial genes. Adding complexity to this process, mitochondrial metabolism and content differs widely among cells, tissues and organs; thereby demanding an extra layer of regulation. While transcription factors bind to DNA in a sequence-dependent manner, transcriptional coactivators interact with them and amplify the activity of the transcriptional machinery by recruiting multi-protein complexes to modify chromatin folding, interact with the RNA polymerase II complex and process messenger RNA [38]. Although the fundamental mechanisms of how mitochondrial biogenesis is orchestrated are still elusive, a major breakthrough came with the discovery of a family of transcriptional coactivators termed PPARγ coactivator 1 (PGC-1) [55]. PGC-1 proteins have emerged as major players in the transcriptional regulatory circuits controlling mitochondrial biogenesis and function.

Conserved across many species, PGC-1 family is formed by three members that share similar domain structures to interact with nuclear receptors [56]. The first and most studied member of this family is PGC-1α. First identified in brown adipose tissue during adaptive thermogenesis—a process that regulates heat production in response to cold and diet, PGC-1α is considered the master regulator of mitochondrial biogenesis in mammals [55]. Similar to PGC-1α, PGC-1β is predominantly expressed in tissues with abundant mitochondria (e.g. heart and skeletal muscle [57]). However, it is not upregulated upon cold exposure [56]. The third member of this family is the PGC-1 related coactivator (PRC). Despite the relatively low homology with the other two isoforms, PRC is ubiquitously expressed and supports mitochondrial biogenesis during early embryogenesis [58, 59]. Together, they bind to and coactivate most of the transcription factors regulating expression of mitochondrial proteins encoded by the nucleus.

Several studies have shown that PGC-1α is capable of regulating virtually every aspect of mitochondrial content [60]. Indeed, by binding to and coactivating NRF-1 and NRF-2, PGC-1α promotes not only a powerful induction of nuclear-encoded mitochondrial respiratory chain subunits, but also leads to the transcription of the mitochondrial genome through the induction of TFAM [61]. Moreover, the interaction between PGC-1α and transcription factors such as PPARα [62], PPARδ [63], ERRα [64], EERγ [64, 65], thyroid hormone receptor [66] and estrogen receptor [57, 67], controls fat and glucose metabolism. And since PGC-1α and PGC-1β share similar molecular structures and functions, it is not surprising that the mitochondrial gene expression driven by these two coactivators overlaps [68, 69]. Interestingly, their work results in mitochondria with different metabolic features [70]; thereby suggesting that distinct upstream pathways modulate PGC-1α and PGC-1β.

The pioneering work of Puigserver and coworkers first showed in 1998 that PGC-1α is dramatically induced (up to 50-fold) upon cold exposure in brown fat and skeletal muscle [55]. Since then, many studies have determined that the expression

of PGC-1 family members are controlled by a variety of external stimuli, such as exercise, cold and nutrient deprivation, in a tissue-dependent manner (reviewed in [71]). Among the transcription factors regulating PGC1-α levels, CREB is responsible for integrating multiple signaling pathways in different cell types to boost mitochondrial function. For example, CREB-dependent induction of PGC1-α occurs in fasted liver [72], in exercised skeletal muscle [73], as well as in brown adipose tissue during cold [55]. Moreover, in a positive autoregulatory loop, PGC-1α regulates its own expression when binding to some of its transcription factors targets such as MEF2 [73] and ERRγ [74]. With equal importance of transcriptional levels, posttranslational modifications of PGC-1α also control mitochondrial biogenesis.

Posttranslational modifications refer to biochemical modifications of a protein (e.g. phosphorylation, acetylation, methylation) capable of influencing not only its structure, but also its activity [75]. The fine-tuning of PGC-1α activity occurs via posttranslational mechanisms. First, phosphorylation of PGC-1α protein is able to triple its half-life, which is relatively short (~2.3 h) [76]. Second, posttranslational modifications interfere with PGC-1α signal transduction by either increasing or inhibiting its activity. In response to bioenergetics imbalance, PGC-1α displays increased activity when phosphorylated by AMP-activated protein kinase (AMPK) [77] and deacetylated by Sirtuin 1 (SIRT1) [78]. On the contrary, PGC-1α can be phosphorylated by glycogen synthase kinase 3β (GSK3β) leading to its inhibition and degradation [79]. Third, most of the signaling pathways conducting these protein modifications have their gene expression regulated by PGC-1 family; thus, reinforcing the feed forward loop [80].

Finally, although the molecular mechanisms are not fully elucidated, it has been demonstrated that posttranslational modifications of PGC-1α result in a preferential induction of biogenesis in a time-, tissue- and subset of mitochondrial genes-dependent manner [78, 81]. This can be explained, at least in part, by the discovery of different splicing variants of PGC-1α: novel truncated PGC-1α (NT-PGC-1α), PGC-1α-β and PGC-1α4. While NT-PGC-1α [82] and PGC-1α-β [83] specifically affect energy metabolism by promoting mitochondrial biogenesis in brown adipose tissue and skeletal muscle, respectively, PGC-1α4 leads to skeletal muscle hypertrophy by regulating a non-mitochondrial gene program [84]. Interestingly, exercise is able to induce and activate all these variants [84, 85].

3 Mitochondrial Dynamics

The high-resolution electron microscopy images of mitochondria, published by Palade [5] and Sjostrand [6] in the 1950s, revealed for the first time the unique ultrastructure of these organelles. Those images also showed a lack of physical connection between mitochondria; thus, suggesting that the organelle was stationary and working independently. Two decades later, descriptions of mega-mitochondria formation in tissues such as liver [86] and skeletal muscle [87] started to question this independency. In the 1990s, advances in electron microscopy along with the development

of mitochondrial-targeted fluorescent proteins allowed the observations that mitochondrial can dynamically rearrange their structure over time [88, 89]. Since then, a complete set of genes driving these morphological changes was discovered (reviewed in [90]) and mitochondrial dynamics has been consolidated as a new area of study in mitochondrial biology.

Mitochondrial dynamics refers to a set of processes including the regulation of mitochondrial morphology and connectivity, as well as their position inside the cells. The mitochondrial ability to reshape, rebuild and redistribute itself is orchestrated by the opposite role of fusion and fission processes [90]. Members of a large family of dynamin guanosine triphosphatases (GTPases) use the hydrolysis of guanosine triphosphate (GTP) to create conformational changes in the mitochondrial membrane that will lead to either the union between two organelles or the division of one mitochondrion in two organelles [91] (Fig. 2).

Despite often viewed as a separate phenomenon, the recycling of mitochondria through mitophagy—a specific form of autophagy, is influenced by mitochondrial

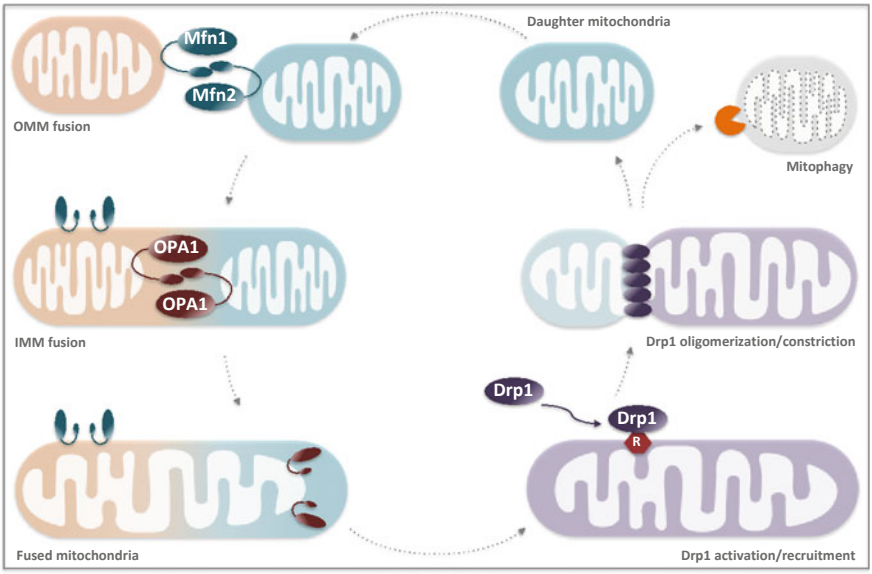


Fig. 2 Simplified model for mitochondrial fusion and fission. The OMM (outer mitochondrial membrane) fuses through interaction of homo- or hereto-oligomers Mfn1 (Mitofusin 1) and Mfn2 (Mitofusin 2) of two opposing mitochondria. Following OMM fusion, OPA1 (Optic atrophy 1) drives IMM (inner mitochondrial membrane) fusion. Please note that, as membrane-bound proteins, Mitofusins and OPA1 are still present in the new fused membranes, but are now disassembled. Mitochondrial fragmentation requires activation of cytosolic Drp1 (Dynamin-related protein 1) and recruitment to the organelle via OMM-bound receptors (R). At these sites, the Drp1 oligomerizes in a ring-like structure and constricts the mitochondria into 2 daughters. Of interest, asymmetrical fission of a damaged or senescent mitochondrion produces 1 dysfunctional organelle that can either be eliminated by mitophagy or re-enter the mitochondrial network and regenerate by fusing with other healthy organelles

fission and therefore directly interferes with the dynamic nature of the organelle. To a detailed description of mitophagy, readers are referred to excellent reviews on this topic [92, 93]. Together, mitochondrial fusion-fission machinery, mitochondrial biogenesis and mitophagy comprise a well-conserved quality control axis capable of controlling the function of the organelle, and as consequence, interfering with cellular physiology [94].

3.1 Mitochondrial Fusion

Mitochondrial fusion is an evolutionary conserved process that merges two neighboring mitochondria. By allowing the exchange of mitochondrial proteins, metabolites and mtDNA, mitochondrial fusion maximizes cellular respiration [95]. It is also required for the maintenance of mtDNA integrity [96]. Moreover, the newly elongated fused organelle prevents erroneous degradation of mitochondria [17]. Considering that mitochondria have outer and inner membranes, the fusion process requires bringing together four membranes in separated events. First, mitofusin 1 (Mfn1) and mitofusin 2 (Mfn2) proteins are responsible for fusing the outer mitochondrial membrane. Later, optic atrophy factor 1 (OPA1) governs the union of the inner mitochondrial membrane [97]. As nuclear-encoded mitochondrial proteins, they need to be synthesized in the cytosol and imported into mitochondria, therefore reinforcing the connection between mitochondrial biogenesis and dynamics. Additionally, these GTPases contain a transmembrane domain that anchors part of them to the lipid bilayer, whereas their free part can physically interact with other GTPases to promote the tethering [98].

Mfn1 and Mfn2 are the major players in promoting outer membrane shape-changes. These isoforms display high homology (~80%) and initiate the fusion of the outer mitochondrial membrane by the formation of homo- (Mfn1-Mfn1 or Mfn2-Mfn2) or hetero-oligomers (Mfn1-Mfn2) between adjacent organelles [99]. Despite widely expressed and essential for embryonic development [100], Mfn1 is more abundant in heart and liver, while Mfn2 predominates in skeletal muscle, brain and adipose tissue [101]. Interestingly, each mitofusin not only differently affects mitochondrial morphology, but also plays distinct roles in cellular physiology. The absence of Mfn1 leads to highly fragmented mitochondria when compared to Mfn2 downregulation [100]. Moreover, Mfn2 also participates in calcium regulation by tethering the mitochondria to the endoplasmic reticulum [102, 103].

In order to complete the fusion process, OPA1 drives the unification of the two inner mitochondrial membranes. This intermembrane space-localized GTPase not only suffers alternative splicing—a mechanism by which different messenger RNA are generated from the same gene, but its activity is also regulated by proteolytic processing [104]. Because of that, there are at least eight variants of OPA1 in humans containing one or two proteolytic sites [105]. The mitochondrial proteases OMA1 and YME1L1 are responsible for generating long and short OPA1 isoforms, in a membrane potential dependent manner [106, 107]. Despite the fact that both

isoforms are required for full fusion events, an excessive processing of short OPA1 limits fusion; therefore, triggering mitochondrial fragmentation [108–110]. Finally, regardless governing the delicate balance between fusion and fission, OPA1 variants are able to control apoptosis by regulating the cristae morphology and consequent release of cytochrome c—a component of ETC that triggers programed cell death [109, 111].

3.2 Mitochondrial Fission

Mitochondrial fission process is responsible for the asymmetrical segregation of portions of the organelle. Whereas this new spherical and smaller organelle facilitates motility throughout the cell, it is also involved in mtDNA replication and inheritance during cellular proliferation [112]. Fragmentation of the mitochondrial network also permits the selective removal of damaged organelles by mitophagy [18]. Unlike mitochondrial fusion, the division of the outer and inner membranes of the organelle is catalyzed by a single GTPase effector—dynamin-related protein 1 (Drp1) [113]. Unlike mitochondrial fusion-related proteins, Drp1 is a nuclear-encoded protein that resides in the cytosol as a small oligomer, thereby demanding recruitment to the mitochondrial surface. The assembly of several Drp1 oligomers forms a ring-like structure around the outer mitochondrial membrane and cut mitochondria into two separate entities in a GTP hydrolysis-dependent manner [114].

A multi-step process is required before completing mitochondrial membrane remodeling. First, Drp1 needs to be activated in order to translocate from the cytosol to mitochondria. Among the posttranslational modifications regulating Drp1 activity, phosphorylation has been extensively studied and serves as an efficient way to synchronize intracellular signaling pathways and mitochondrial metabolism. For example, protein kinase A (PKA) phosphorylation of Drp1 at serine-637 blocks fission and protects mitochondrial from degradation during starvation [115]. Dephosphorylation of the same residue by the phosphatase calcineurin triggers fission in a calcium-induced mitochondrial dysfunction environment [116, 117]. Ubiquitination of Drp1 by E3 ligases can either induce mitochondrial fragmentation or inhibit fission by promoting Drp1 degradation [118, 119]. Additional posttranslational modifications of Drp1 (e.g. SUMOylation and S-nitrosylation) also dictate mitochondrial dynamics. These regulatory mechanisms are reviewed elsewhere [120, 121].

Once activated, the second step involves the recruitment of Drp1 to specific regions of the outer mitochondrial membrane. Four specific adaptor proteins, also termed Drp1 receptors, facilitate this anchoring process: mitochondrial fission 1 protein (Fis1), mitochondrial fission factor (Mff) and mitochondrial dynamics proteins of 49 and 51 kDa (MiD49 and MiD51, respectively) [113, 122, 123]. This receptor-mediated recruitment of Drp1 assists mitochondrial fragmentation by allowing the self-assemble of Drp1 into oligomeric complexes at specific sites of the outer membrane pre-constricted by the endoplasmic reticulum [124, 125]. Similar to Drp1, these receptors can be activated by posttranslational modifications. In particular, MFF

can be phosphorylated by AMPK in response to nutrient excess, favoring mitochondrial fission [126, 127]. Finally, recent evidence place another GTPase—dynamin 2 (Dyn2), as a mechanoenzyme involved in terminating membrane scission. It has been proposed that Drp1-mediated constriction allows Dyn2 assembly to complete the fission event [128].

4 Mitochondrial Dynamics in Health and Disease

Given the fact that mitochondrial biogenesis and dynamics interfere with a variety of intracellular processes including ATP production, ROS release and apoptosis, it is not surprising that they are critical in the context of both physiological and pathological events [129]. Disruption of mitochondrial homeostasis follows the clinical progression of a variety of chronic degenerative diseases (e.g. Heart Failure and Diabetes) [130]. Moreover, mitochondrial dysfunction is a common feature of rare inherited mitochondrial diseases, which are driven by mutations in either nuclear or mitochondrial DNA (e.g. Leigh syndrome and Friedreich's ataxia) [131]. Either way, the inability of maintaining a healthy mitochondrial population has been placed as a central determinant of several diseases. Here, we discuss mitochondrial biogenesis and dynamics in the context of cardiac, metabolic and neurodegenerative diseases, as well as in mitochondrial diseases.

Since heart contractility requires elevated and sustained levels of ATP, an overall failure of mitochondrial function has been placed as a hallmark of cardiac diseases [132, 133]. Disrupted mitochondrial morphology—characterized by increase number of smaller organelles [134], has been detected in cardiac patients suggesting imbalance between fusion and fission as critical factor for heart pathophysiology. Indeed, while absence of Mfn1 or Mfn2 [135–137], or excessive OPA1 cleavage [138] are sufficient to disrupt mitochondrial fusion leading to cardiomyopathy in mice, inactivation of Drp1 blunts excessive fission of the organelle, thus counteracting cardiac dysfunction [139]. Likewise, small molecules capable of blocking fission (i.e. Mdivi-1 and P110) [139, 140] or improving fusion (SAMβA) [141], as well as exercise [142], reestablish mitochondrial dynamics and improve clinical outcome in preclinical models of cardiac diseases. Of interest, failing hearts display loss of mtDNA along with reduced expression of mitochondrial biogenesis markers [143]. Moreover, cardiac specific ablation of PGC-1α leads to cardiac dysfunction in mice [144]. Because of that, activators of AMPK (i.e. Metformin, AICAR) capable of stimulating mitochondrial biogenesis [145], are emerging as promising therapies to treat cardiovascular diseases [146].

Metabolic disorders including type 2 Diabetes and obesity not only arises from a complex combination of genetic and environmental factors such as insulin resistance, dyslipidemia, erroneous food intake and physical inactivity [147], but also display mitochondrial dysfunction as a common feature [148]. Part of this phenotype is due to impaired mitochondrial biogenesis and dynamics in a wide spectrum of tissues. Reduced expression of PGC-1 members along with defective translation of genes

encoding subunits of respiratory chain have been observed in skeletal muscle from diabetic patients [149] and adipose tissue from obese subjects [150]. Strengthening these results, mice lacking PGC-1α in adipose tissue develop insulin resistance and abnormal thermogenic response [151]. Moreover, mitochondrial biogenesis have been linked to the beneficial effects of agonists of AMPK [152, 153] and PPAR [154, 155]—widely used drugs for the treatment of type 2 Diabetes. The excessive nutrient environment observed in metabolic disorders also promotes disruption of mitochondrial dynamics. Consistent with reduction of Mfn2 levels [156, 157], mitochondrial fragmentation associated with insulin sensitivity and altered metabolism has been observed in obesity and type 2 Diabetes [15, 158].

Along with progressive loss of neuronal systems, disruption of mitochondrial homeostasis plays a role in the pathogenesis of neurodegenerative disorders such as Parkinson´s, Alzheimer´s and Huntington´s diseases [159, 160]. Analysis of human brains from Alzheimer´s patients revealed not only structurally abnormal mitochondria [161, 162], but also indicated a strong link between Drp1-mediated mitochondrial fission and neurodegeneration [163]. Indeed, blocking mitochondrial fragmentation exhibits beneficial effects in preclinical models of Huntington´s [164] and Parkinson's [165] disease, and Amyotrophic lateral sclerosis [166]. On the contrary, loss-of-function Drp1 mutations leading to giant and aberrant mitochondria are often associated with lethal neurological disorders including microcephaly [167] and refractory epilepsy [168]; therefore, reinforcing the role of an exquisite balance of mitochondrial fusion and fission events in cellular physiology. In the context of mitochondrial biogenesis, deficiencies in the ETC are related to mtDNA mutations in Alzheimer's patients, which suppress mitochondrial transcription and replication [169]. Similarly, studies in animals have shown that whereas impaired mitochondrial biogenesis leads to loss of neurons [170], PGC-1α upregulation protects neural cells against oxidative stress-induced death [171].

Mostly driven by loss-of-function mutations in mtDNA or nuclear DNA, mitochondrial diseases refer to a heterogeneous group of disorders triggered by mitochondrial dysfunction [172]. Regardless the disease etiology, there is an overall decrease in content and function of respiratory chain subunits [173–175]. In this context, PGC-1α overexpression can boost ATP production by increasing the amount of the organelle in Leigh syndrome [174]. Inducers of mitochondrial biogenesis (i.e. AICAR) also delay the progression of mitochondrial myopathies in mice [174, 176]. Despite the fact that most of these disorders arise from defects in OXPHOS components, progressive neuronal degeneration along with aberrant mitochondrial morphology are observed in preclinical models of Leigh syndrome [175]. Progressive loss of vision observed in autosomal dominant optic atrophy disease is associated with OPA1 mutations [177]. Moreover, impaired mitochondrial fusion or fission by mutations in Mfn2 [178] and Dyn2 genes [179] cause the inherited Charcot Marie Tooth disease. Of interest, due to its involvement in mtDNA replication [112, 180], disruption of mitochondrial dynamics may increase the susceptibility to these inborn errors. Finally, highlighting the dynamic nature of mitochondria, gene therapy is the latest and attractive strategy to restore mitochondrial function and counteract clinical progression of primary mitochondrial diseases [181–184].

5 Concluding Remarks

The dynamic behavior of mitochondria morphology, controlled by mitochondrial biogenesis and fission-fusion machineries, are determinant for the whole-body homeostasis. Fluctuations in mitochondrial quantity, size and cellular position occur in response to numerous stress and metabolic conditions, which will lead to divergent outcomes. If transient, perturbations of mitochondrial mass and morphology enable metabolic adaptations to meet energetic requirements. On the contrary, sustained stress-induced mitochondrial dysfunction often triggers mitochondrial fragmentation and induces cell death. Moreover, due to the dynamic nature of mitochondria, studying the physiological and pathological significance of mitochondrial network in a time-, tissue- and stress-dependent manner is a challenging task. The development of advanced techniques capable of tracking fusion and fission events in vivo, as well as the identification of new players controlling biogenesis and dynamics will be crucial not only to overcome these obstacles, but also to open up new avenues for pharmacological interventions.

References

1. Mitchell P, Moyle J (1967) Chemiosmotic hypothesis of oxidative phosphorylation. Nature 213(5072):137–139
2. Sagan L (1967) On the origin of mitosing cells. J Theor Biol 14(3):255–274
3. Mills EL, Kelly B, O'Neill LAJ (2017) Mitochondria are the powerhouses of immunity. Nat Immunol 18(5):488–498
4. Roger AJ, Munoz-Gomez SA, Kamikawa R (2017) The origin and diversification of mitochondria. Curr Biol 27(21):R1177–R1192
5. Palade GE (1953) An electron microscope study of the mitochondrial structure. J Histochem Cytochem 1(4):188–211
6. Sjostrand FS (1953) Electron microscopy of mitochondria and cytoplasmic double membranes. Nature 171(4340):30–32
7. Boveris A, Chance B (1973) The mitochondrial generation of hydrogen peroxide. General properties and effect of hyperbaric oxygen. Biochem J 134(3):707–716
8. Boveris A, Oshino N, Chance B (1972) The cellular production of hydrogen peroxide. Biochem J 128(3):617–630
9. Walkenstein SS, Weinhouse S (1953) Oxidation of aldehydes by mitochondria of rat tissues. J Biol Chem 200(2):515–523
10. Rafael J, Klaas D, Hohorst HJ (1968) Mitochondria from brown fat: enzymes and respiratory chain phosphorylation during the pre- and postnatal development of the interscapular fat body of the guinea pig. Hoppe Seylers Z Physiol Chem 349(12):1711–1724
11. Rossi CS, Lehninger AL (1964) Stoichiometry of respiratory stimulation, accumulation of Ca++ and phosphate, and oxidative phosphorylation in rat liver mitochondria. J Biol Chem 239:3971–3980
12. Kerr JF, Wyllie AH, Currie AR (1972) Apoptosis: a basic biological phenomenon with wide-ranging implications in tissue kinetics. Br J Cancer 26(4):239–257
13. Rabinowitz M, Swift H (1970) Mitochondrial nucleic acids and their relation to the biogenesis of mitochondria. Physiol Rev 50(3):376–427
14. Bereiter-Hahn J (1990) Behavior of mitochondria in the living cell. Int Rev Cytol 122:1–63

15. Liesa M, Shirihai OS (2013) Mitochondrial dynamics in the regulation of nutrient utilization and energy expenditure. Cell Metab 17(4):491–506
16. Lin J, Handschin C, Spiegelman BM (2005) Metabolic control through the PGC-1 family of transcription coactivators. Cell Metab 1(6):361–370
17. Chen H, Chomyn A, Chan DC (2005) Disruption of fusion results in mitochondrial heterogeneity and dysfunction. J Biol Chem 280(28):26185–26192
18. Twig G, Elorza A, Molina AJ, Mohamed H, Wikstrom JD, Walzer G et al (2008) Fission and selective fusion govern mitochondrial segregation and elimination by autophagy. EMBO J 27(2):433–446
19. Pearce EL, Pearce EJ (2013) Metabolic pathways in immune cell activation and quiescence. Immunity 38(4):633–643
20. Mohanty A, Tiwari-Pandey R, Pandey NR (2019) Mitochondria: the indispensable players in innate immunity and guardians of the inflammatory response. J Cell Commun Signal. 13(3):303–318
21. Zhang Q, Raoof M, Chen Y, Sumi Y, Sursal T, Junger W et al (2010) Circulating mitochondrial DAMPs cause inflammatory responses to injury. Nature 464(7285):104–107
22. Rambold AS, Pearce EL (2018) Mitochondrial dynamics at the interface of immune cell metabolism and function. Trends Immunol 39(1):6–18
23. Robin ED, Wong R (1988) Mitochondrial DNA molecules and virtual number of mitochondria per cell in mammalian cells. J Cell Physiol 136(3):507–513
24. Andersson SG, Zomorodipour A, Andersson JO, Sicheritz-Ponten T, Alsmark UC, Podowski RM et al (1998) The genome sequence of Rickettsia prowazekii and the origin of mitochondria. Nature 396(6707):133–140
25. Calvo SE, Clauser KR, Mootha VK (2016) MitoCarta2.0: an updated inventory of mammalian mitochondrial proteins. Nucleic Acids Res 44(D1):D1251–D1257
26. Pagliarini DJ, Calvo SE, Chang B, Sheth SA, Vafai SB, Ong SE et al (2008) A mitochondrial protein compendium elucidates complex I disease biology. Cell 134(1):112–123
27. Letts JA, Sazanov LA (2017) Clarifying the supercomplex: the higher-order organization of the mitochondrial electron transport chain. Nat Struct Mol Biol 24(10):800–808
28. Garesse R, Vallejo CG (2001) Animal mitochondrial biogenesis and function: a regulatory cross-talk between two genomes. Gene 263(1–2):1–16
29. Jovaisaite V, Auwerx J (2015) The mitochondrial unfolded protein response-synchronizing genomes. Curr Opin Cell Biol 33:74–81
30. Weissbach A, Baltimore D, Bollum F, Gallo R, Korn D (1975) Nomenclature of eukaryotic DNA polymerases. Science 190(4212):401–402
31. Yasukawa T, Kang D (2018) An overview of mammalian mitochondrial DNA replication mechanisms. J Biochem 164(3):183–193
32. D'Souza AR, Minczuk M (2018) Mitochondrial transcription and translation: overview. Essays Biochem 62(3):309–320
33. Vaquerizas JM, Kummerfeld SK, Teichmann SA, Luscombe NM (2009) A census of human transcription factors: function, expression and evolution. Nat Rev Genet 10(4):252–263
34. Evans MJ, Scarpulla RC (1989) Interaction of nuclear factors with multiple sites in the somatic cytochrome c promoter. Characterization of upstream NRF-1, ATF, and intron Sp1 recognition sequences. J Biol Chem 264(24):14361–8.
35. Evans MJ, Scarpulla RC (1990) NRF-1: a trans-activator of nuclear-encoded respiratory genes in animal cells. Genes Dev 4(6):1023–1034
36. Virbasius JV, Virbasius CA, Scarpulla RC (1993) Identity of GABP with NRF-2, a multisubunit activator of cytochrome oxidase expression, reveals a cellular role for an ETS domain activator of viral promoters. Genes Dev 7(3):380–392
37. Itoh K, Chiba T, Takahashi S, Ishii T, Igarashi K, Katoh Y et al (1997) An Nrf2/small Maf heterodimer mediates the induction of phase II detoxifying enzyme genes through antioxidant response elements. Biochem Biophys Res Commun 236(2):313–322
38. Kelly DP, Scarpulla RC (2004) Transcriptional regulatory circuits controlling mitochondrial biogenesis and function. Genes Dev 18(4):357–368

39. Blesa JR, Hernandez JM, Hernandez-Yago J (2004) NRF-2 transcription factor is essential in promoting human Tomm70 gene expression. Mitochondrion 3(5):251–259
40. Virbasius JV, Scarpulla RC (1994) Activation of the human mitochondrial transcription factor A gene by nuclear respiratory factors: a potential regulatory link between nuclear and mitochondrial gene expression in organelle biogenesis. Proc Natl Acad Sci U S A 91(4):1309–1313
41. Gleyzer N, Vercauteren K, Scarpulla RC (2005) Control of mitochondrial transcription specificity factors (TFB1M and TFB2M) by nuclear respiratory factors (NRF-1 and NRF-2) and PGC-1 family coactivators. Mol Cell Biol 25(4):1354–1366
42. Larsson NG, Wang J, Wilhelmsson H, Oldfors A, Rustin P, Lewandoski M et al (1998) Mitochondrial transcription factor A is necessary for mtDNA maintenance and embryogenesis in mice. Nat Genet 18(3):231–236
43. Falkenberg M, Gaspari M, Rantanen A, Trifunovic A, Larsson NG, Gustafsson CM (2002) Mitochondrial transcription factors B1 and B2 activate transcription of human mtDNA. Nat Genet 31(3):289–294
44. Piantadosi CA, Carraway MS, Babiker A, Suliman HB (2008) Heme oxygenase-1 regulates cardiac mitochondrial biogenesis via Nrf2-mediated transcriptional control of nuclear respiratory factor-1. Circ Res 103(11):1232–1240
45. Huo L, Scarpulla RC (2001) Mitochondrial DNA instability and peri-implantation lethality associated with targeted disruption of nuclear respiratory factor 1 in mice. Mol Cell Biol 21(2):644–654
46. Chen H, Hu Y, Fang Y, Djukic Z, Yamamoto M, Shaheen NJ et al (2014) Nrf2 deficiency impairs the barrier function of mouse oesophageal epithelium. Gut 63(5):711–719
47. Issemann I, Green S (1990) Activation of a member of the steroid hormone receptor superfamily by peroxisome proliferators. Nature 347(6294):645–650
48. Schmidt A, Endo N, Rutledge SJ, Vogel R, Shinar D, Rodan GA (1992) Identification of a new member of the steroid hormone receptor superfamily that is activated by a peroxisome proliferator and fatty acids. Mol Endocrinol 6(10):1634–1641
49. Greene ME, Blumberg B, McBride OW, Yi HF, Kronquist K, Kwan K et al (1995) Isolation of the human peroxisome proliferator activated receptor gamma cDNA: expression in hematopoietic cells and chromosomal mapping. Gene Expr 4(4–5):281–299
50. Madrazo JA, Kelly DP (2008) The PPAR trio: regulators of myocardial energy metabolism in health and disease. J Mol Cell Cardiol 44(6):968–975
51. Bookout AL, Jeong Y, Downes M, Yu RT, Evans RM, Mangelsdorf DJ (2006) Anatomical profiling of nuclear receptor expression reveals a hierarchical transcriptional network. Cell 126(4):789–799
52. Eichner LJ, Giguere V (2011) Estrogen related receptors (ERRs): a new dawn in transcriptional control of mitochondrial gene networks. Mitochondrion 11(4):544–552
53. Huss JM, Torra IP, Staels B, Giguere V, Kelly DP (2004) Estrogen-related receptor alpha directs peroxisome proliferator-activated receptor alpha signaling in the transcriptional control of energy metabolism in cardiac and skeletal muscle. Mol Cell Biol 24(20):9079–9091
54. Scarpulla RC, Vega RB, Kelly DP (2012) Transcriptional integration of mitochondrial biogenesis. Trends Endocrinol Metab 23(9):459–466
55. Puigserver P, Wu Z, Park CW, Graves R, Wright M, Spiegelman BM (1998) A cold-inducible coactivator of nuclear receptors linked to adaptive thermogenesis. Cell 92(6):829–839
56. Lin J, Puigserver P, Donovan J, Tarr P, Spiegelman BM (2002) Peroxisome proliferator-activated receptor gamma coactivator 1beta (PGC-1beta), a novel PGC-1-related transcription coactivator associated with host cell factor. J Biol Chem 277(3):1645–1648
57. Knutti D, Kaul A, Kralli A (2000) A tissue-specific coactivator of steroid receptors, identified in a functional genetic screen. Mol Cell Biol 20(7):2411–2422
58. Andersson U, Scarpulla RC (2001) Pgc-1-related coactivator, a novel, serum-inducible coactivator of nuclear respiratory factor 1-dependent transcription in mammalian cells. Mol Cell Biol 21(11):3738–3749

59. He X, Sun C, Wang F, Shan A, Guo T, Gu W et al (2012) Peri-implantation lethality in mice lacking the PGC-1-related coactivator protein. Dev Dyn 241(5):975–983
60. Finck BN, Kelly DP (2006) PGC-1 coactivators: inducible regulators of energy metabolism in health and disease. J Clin Invest 116(3):615–622
61. Wu Z, Puigserver P, Andersson U, Zhang C, Adelmant G, Mootha V et al (1999) Mechanisms controlling mitochondrial biogenesis and respiration through the thermogenic coactivator PGC-1. Cell 98(1):115–124
62. Vega RB, Huss JM, Kelly DP (2000) The coactivator PGC-1 cooperates with peroxisome proliferator-activated receptor alpha in transcriptional control of nuclear genes encoding mitochondrial fatty acid oxidation enzymes. Mol Cell Biol 20(5):1868–1876
63. Wang YX, Lee CH, Tiep S, Yu RT, Ham J, Kang H et al (2003) Peroxisome-proliferator-activated receptor delta activates fat metabolism to prevent obesity. Cell 113(2):159–170
64. Schreiber SN, Knutti D, Brogli K, Uhlmann T, Kralli A (2003) The transcriptional coactivator PGC-1 regulates the expression and activity of the orphan nuclear receptor estrogen-related receptor alpha (ERRalpha). J Biol Chem 278(11):9013–9018
65. Huss JM, Kopp RP, Kelly DP (2002) Peroxisome proliferator-activated receptor coactivator-1alpha (PGC-1alpha) coactivates the cardiac-enriched nuclear receptors estrogen-related receptor-alpha and -gamma. Identification of novel leucine-rich interaction motif within PGC-1alpha. J Biol Chem 277(43):40265–40274
66. Zhang Y, Ma K, Song S, Elam MB, Cook GA, Park EA (2004) Peroxisomal proliferator-activated receptor-gamma coactivator-1 alpha (PGC-1 alpha) enhances the thyroid hormone induction of carnitine palmitoyltransferase I (CPT-I alpha). J Biol Chem 279(52):53963–53971
67. Tcherepanova I, Puigserver P, Norris JD, Spiegelman BM, McDonnell DP (2000) Modulation of estrogen receptor-alpha transcriptional activity by the coactivator PGC-1. J Biol Chem 275(21):16302–16308
68. Lai L, Leone TC, Zechner C, Schaeffer PJ, Kelly SM, Flanagan DP et al (2008) Transcriptional coactivators PGC-1alpha and PGC-lbeta control overlapping programs required for perinatal maturation of the heart. Genes Dev 22(14):1948–1961
69. Espinoza DO, Boros LG, Crunkhorn S, Gami H, Patti ME (2010) Dual modulation of both lipid oxidation and synthesis by peroxisome proliferator-activated receptor-gamma coactivator-1alpha and -1beta in cultured myotubes. FASEB J 24(4):1003–1014
70. St-Pierre J, Lin J, Krauss S, Tarr PT, Yang R, Newgard CB et al (2003) Bioenergetic analysis of peroxisome proliferator-activated receptor gamma coactivators 1alpha and 1beta (PGC-1alpha and PGC-1beta) in muscle cells. J Biol Chem 278(29):26597–26603
71. Handschin C, Spiegelman BM (2006) Peroxisome proliferator-activated receptor gamma coactivator 1 coactivators, energy homeostasis, and metabolism. Endocr Rev 27(7):728–735
72. Herzig S, Long F, Jhala US, Hedrick S, Quinn R, Bauer A et al (2001) CREB regulates hepatic gluconeogenesis through the coactivator PGC-1. Nature 413(6852):179–183
73. Handschin C, Rhee J, Lin J, Tarr PT, Spiegelman BM (2003) An autoregulatory loop controls peroxisome proliferator-activated receptor gamma coactivator 1alpha expression in muscle. Proc Natl Acad Sci U S A 100(12):7111–7116
74. Wang L, Liu J, Saha P, Huang J, Chan L, Spiegelman B et al (2005) The orphan nuclear receptor SHP regulates PGC-1alpha expression and energy production in brown adipocytes. Cell Metab 2(4):227–238
75. Santos AL, Lindner AB (2017) Protein posttranslational modifications: roles in aging and age-related disease. Oxid Med Cell Longev 2017:5716409
76. Knutti D, Kressler D, Kralli A (2001) Regulation of the transcriptional coactivator PGC-1 via MAPK-sensitive interaction with a repressor. Proc Natl Acad Sci U S A 98(17):9713–9718
77. Zong H, Ren JM, Young LH, Pypaert M, Mu J, Birnbaum MJ et al (2002) AMP kinase is required for mitochondrial biogenesis in skeletal muscle in response to chronic energy deprivation. Proc Natl Acad Sci U S A 99(25):15983–15987
78. Rodgers JT, Lerin C, Haas W, Gygi SP, Spiegelman BM, Puigserver P (2005) Nutrient control of glucose homeostasis through a complex of PGC-1alpha and SIRT1. Nature 434(7029):113–118

79. Anderson RM, Barger JL, Edwards MG, Braun KH, O'Connor CE, Prolla TA et al (2008) Dynamic regulation of PGC-1alpha localization and turnover implicates mitochondrial adaptation in calorie restriction and the stress response. Aging Cell 7(1):101–111
80. Fernandez-Marcos PJ, Auwerx J (2011) Regulation of PGC-1alpha, a nodal regulator of mitochondrial biogenesis. Am J Clin Nutr 93(4):884S-S890
81. Winder WW, Holmes BF, Rubink DS, Jensen EB, Chen M, Holloszy JO (1985) Activation of AMP-activated protein kinase increases mitochondrial enzymes in skeletal muscle. J Appl Physiol 88(6):2219–2226
82. Zhang Y, Huypens P, Adamson AW, Chang JS, Henagan TM, Boudreau A et al (2009) Alternative mRNA splicing produces a novel biologically active short isoform of PGC-1alpha. J Biol Chem 284(47):32813–32826
83. Tadaishi M, Miura S, Kai Y, Kano Y, Oishi Y, Ezaki O (2011) Skeletal muscle-specific expression of PGC-1alpha-b, an exercise-responsive isoform, increases exercise capacity and peak oxygen uptake. PLoS One 6(12):e28290
84. Ruas JL, White JP, Rao RR, Kleiner S, Brannan KT, Harrison BC et al (2012) A PGC-1alpha isoform induced by resistance training regulates skeletal muscle hypertrophy. Cell 151(6):1319–1331
85. Ydfors M, Fischer H, Mascher H, Blomstrand E, Norrbom J, Gustafsson T (2013) The truncated splice variants, NT-PGC-1alpha and PGC-1alpha4, increase with both endurance and resistance exercise in human skeletal muscle. Physiol Rep 1(6):e00140
86. Asano M, Wakabayashi T, Ishikawa K, Kishimoto H (1977) Induction of megamitochondria in mouse hepatocytes by nialamide. J Electron Microsc (Tokyo) 26(2):141–144
87. Bakeeva LE, Chentsov YuS, Skulachev VP (1978) Mitochondrial framework (reticulum mitochondriale) in rat diaphragm muscle. Biochim Biophys Acta 501(3):349–369
88. Bereiter-Hahn J, Voth M (1994) Dynamics of mitochondria in living cells: shape changes, dislocations, fusion, and fission of mitochondria. Microsc Res Tech 27(3):198–219
89. Cortese JD (1998) Stimulation of rat liver mitochondrial fusion by an outer membrane-derived aluminum fluoride-sensitive protein fraction. Exp Cell Res 240(1):122–133
90. Liesa M, Palacin M, Zorzano A (2009) Mitochondrial dynamics in mammalian health and disease. Physiol Rev 89(3):799–845
91. Ishihara N, Otera H, Oka T, Mihara K (2013) Regulation and physiologic functions of GTPases in mitochondrial fusion and fission in mammals. Antioxid Redox Signal 19(4):389–399
92. Youle RJ, Narendra DP (2011) Mechanisms of mitophagy. Nat Rev Mol Cell Biol 12(1):9–14
93. Hamacher-Brady A, Brady NR (2016) Mitophagy programs: mechanisms and physiological implications of mitochondrial targeting by autophagy. Cell Mol Life Sci 73(4):775–795
94. Eisner V, Picard M, Hajnoczky G (2018) Mitochondrial dynamics in adaptive and maladaptive cellular stress responses. Nat Cell Biol 20(7):755–765
95. Rambold AS, Kostelecky B, Elia N, Lippincott-Schwartz J (2011) Tubular network formation protects mitochondria from autophagosomal degradation during nutrient starvation. Proc Natl Acad Sci U S A 108(25):10190–10195
96. Chen H, Vermulst M, Wang YE, Chomyn A, Prolla TA, McCaffery JM et al (2010) Mitochondrial fusion is required for mtDNA stability in skeletal muscle and tolerance of mtDNA mutations. Cell 141(2):280–289
97. Malka F, Guillery O, Cifuentes-Diaz C, Guillou E, Belenguer P, Lombes A et al (2005) Separate fusion of outer and inner mitochondrial membranes. EMBO Rep 6(9):853–859
98. Koshiba T, Detmer SA, Kaiser JT, Chen H, McCaffery JM, Chan DC (2004) Structural basis of mitochondrial tethering by mitofusin complexes. Science 305(5685):858–862
99. Ishihara N, Eura Y, Mihara K (2004) Mitofusin 1 and 2 play distinct roles in mitochondrial fusion reactions via GTPase activity. J Cell Sci 117(Pt 26):6535–6546
100. Chen H, Detmer SA, Ewald AJ, Griffin EE, Fraser SE, Chan DC (2003) Mitofusins Mfn1 and Mfn2 coordinately regulate mitochondrial fusion and are essential for embryonic development. J Cell Biol 160(2):189–200
101. Eura Y, Ishihara N, Yokota S, Mihara K (2003) Two mitofusin proteins, mammalian homologues of FZO, with distinct functions are both required for mitochondrial fusion. J Biochem 134(3):333–344

102. de Brito OM, Scorrano L (2008) Mitofusin 2 tethers endoplasmic reticulum to mitochondria. Nature 456(7222):605–610
103. Naon D, Zaninello M, Giacomello M, Varanita T, Grespi F, Lakshminaranayan S et al (2016) Critical reappraisal confirms that Mitofusin 2 is an endoplasmic reticulum-mitochondria tether. Proc Natl Acad Sci U S A 113(40):11249–11254
104. Del Dotto V, Fogazza M, Carelli V, Rugolo M, Zanna C (2018) Eight human OPA1 isoforms, long and short: what are they for? Biochim Biophys Acta Bioenerg 1859(4):263–269
105. Delettre C, Griffoin JM, Kaplan J, Dollfus H, Lorenz B, Faivre L et al (2001) Mutation spectrum and splicing variants in the OPA1 gene. Hum Genet 109(6):584–591
106. Song Z, Chen H, Fiket M, Alexander C, Chan DC (2007) OPA1 processing controls mitochondrial fusion and is regulated by mRNA splicing, membrane potential, and Yme1L. J Cell Biol 178(5):749–755
107. Ehses S, Raschke I, Mancuso G, Bernacchia A, Geimer S, Tondera D et al (2009) Regulation of OPA1 processing and mitochondrial fusion by m-AAA protease isoenzymes and OMA1. J Cell Biol 187(7):1023–1036
108. Anand R, Wai T, Baker MJ, Kladt N, Schauss AC, Rugarli E et al (2014) The i-AAA protease YME1L and OMA1 cleave OPA1 to balance mitochondrial fusion and fission. J Cell Biol 204(6):919–929
109. Ishihara N, Fujita Y, Oka T, Mihara K (2006) Regulation of mitochondrial morphology through proteolytic cleavage of OPA1. EMBO J 25(13):2966–2977
110. Head B, Griparic L, Amiri M, Gandre-Babbe S, van der Bliek AM (2009) Inducible proteolytic inactivation of OPA1 mediated by the OMA1 protease in mammalian cells. J Cell Biol 187(7):959–966
111. Olichon A, Baricault L, Gas N, Guillou E, Valette A, Belenguer P et al (2003) Loss of OPA1 perturbates the mitochondrial inner membrane structure and integrity, leading to cytochrome c release and apoptosis. J Biol Chem 278(10):7743–7746
112. Mishra P, Chan DC (2014) Mitochondrial dynamics and inheritance during cell division, development and disease. Nat Rev Mol Cell Biol 15(10):634–646
113. Gandre-Babbe S, van der Bliek AM (2008) The novel tail-anchored membrane protein Mff controls mitochondrial and peroxisomal fission in mammalian cells. Mol Biol Cell 19(6):2402–2412
114. Smirnova E, Griparic L, Shurland DL, van der Bliek AM (2001) Dynamin-related protein Drp1 is required for mitochondrial division in mammalian cells. Mol Biol Cell 12(8):2245–2256
115. Gomes LC, Di Benedetto G, Scorrano L (2011) During autophagy mitochondria elongate, are spared from degradation and sustain cell viability. Nat Cell Biol 13(5):589–598
116. Cereghetti GM, Stangherlin A, Martins de Brito O, Chang CR, Blackstone C, Bernardi P, et al (2008) Dephosphorylation by calcineurin regulates translocation of Drp1 to mitochondria. Proc Natl Acad Sci U S A 105(41):15803–15808
117. Cribbs JT, Strack S (2007) Reversible phosphorylation of Drp1 by cyclic AMP-dependent protein kinase and calcineurin regulates mitochondrial fission and cell death. EMBO Rep 8(10):939–944
118. Yonashiro R, Ishido S, Kyo S, Fukuda T, Goto E, Matsuki Y et al (2006) A novel mitochondrial ubiquitin ligase plays a critical role in mitochondrial dynamics. EMBO J 25(15):3618–3626
119. Poole AC, Thomas RE, Andrews LA, McBride HM, Whitworth AJ, Pallanck LJ (2008) The PINK1/Parkin pathway regulates mitochondrial morphology. Proc Natl Acad Sci U S A 105(5):1638–1643
120. Chang CR, Blackstone C (2010) Dynamic regulation of mitochondrial fission through modification of the dynamin-related protein Drp1. Ann N Y Acad Sci 1201:34–39
121. Benard G, Karbowski M (2009) Mitochondrial fusion and division: regulation and role in cell viability. Semin Cell Dev Biol 20(3):365–374
122. Loson OC, Song Z, Chen H, Chan DC (2013) Fis1, Mff, MiD49, and MiD51 mediate Drp1 recruitment in mitochondrial fission. Mol Biol Cell 24(5):659–667
123. Palmer CS, Osellame LD, Laine D, Koutsopoulos OS, Frazier AE, Ryan MT (2011) MiD49 and MiD51, new components of the mitochondrial fission machinery. EMBO Rep 12(6):565–573

124. Friedman JR, Lackner LL, West M, DiBenedetto JR, Nunnari J, Voeltz GK (2011) ER tubules mark sites of mitochondrial division. Science 334(6054):358–362
125. Ji WK, Chakrabarti R, Fan X, Schoenfeld L, Strack S, Higgs HN (2017) Receptor-mediated Drp1 oligomerization on endoplasmic reticulum. J Cell Biol 216(12):4123–4139
126. Toyama EQ, Herzig S, Courchet J, Lewis TL, Jr., Loson OC, Hellberg K, et al (2016) Metabolism. AMP-activated protein kinase mediates mitochondrial fission in response to energy stress. Science 351(6270):275–281
127. Ducommun S, Deak M, Sumpton D, Ford RJ, Nunez Galindo A, Kussmann M et al (2015) Motif affinity and mass spectrometry proteomic approach for the discovery of cellular AMPK targets: identification of mitochondrial fission factor as a new AMPK substrate. Cell Signal 27(5):978–988
128. Lee JE, Westrate LM, Wu H, Page C, Voeltz GK (2016) Multiple dynamin family members collaborate to drive mitochondrial division. Nature 540(7631):139–143
129. Nunnari J, Suomalainen A (2012) Mitochondria: in sickness and in health. Cell 148(6):1145–1159
130. Sorrentino V, Romani M, Mouchiroud L, Beck JS, Zhang H, D'Amico D et al (2017) Enhancing mitochondrial proteostasis reduces amyloid-beta proteotoxicity. Nature 552(7684):187–193
131. Pfeffer G, Majamaa K, Turnbull DM, Thorburn D, Chinnery PF (2012) Treatment for mitochondrial disorders. Cochrane Database Syst Rev (4):CD004426
132. Campos JC, Bozi LH, Bechara LR, Lima VM, Ferreira JC (2016) Mitochondrial Quality Control in Cardiac Diseases. Front Physiol 7:479
133. Campos JC, Gomes KM, Ferreira JC (2013) Impact of exercise training on redox signaling in cardiovascular diseases. Food Chem Toxicol 62:107–119
134. Nan J, Zhu W, Rahman MS, Liu M, Li D, Su S et al (2017) Molecular regulation of mitochondrial dynamics in cardiac disease. Biochim Biophys Acta Mol Cell Res 1864(7):1260–1273
135. Song M, Franco A, Fleischer JA, Zhang L, Dorn GW (2017) Abrogating mitochondrial dynamics in mouse hearts accelerates mitochondrial senescence (2nd edn). Cell Metab 26(6):872–883, e5
136. Chen Y, Dorn GW 2nd (2013) PINK1-phosphorylated mitofusin 2 is a Parkin receptor for culling damaged mitochondria. Science 340(6131):471–475
137. Chen Y, Liu Y, Dorn GW 2nd (2011) Mitochondrial fusion is essential for organelle function and cardiac homeostasis. Circ Res 109(12):1327–1331
138. Wai T, Garcia-Prieto J, Baker MJ, Merkwirth C, Benit P, Rustin P, et al (2015) Imbalanced OPA1 processing and mitochondrial fragmentation cause heart failure in mice. Science 350(6265):aad0116
139. Disatnik MH, Ferreira JC, Campos JC, Gomes KS, Dourado PM, Qi X, et al (2013) Acute inhibition of excessive mitochondrial fission after myocardial infarction prevents long-term cardiac dysfunction. J Am Heart Assoc 2(5):e000461
140. Givvimani S, Munjal C, Tyagi N, Sen U, Metreveli N, Tyagi SC (2012) Mitochondrial division/mitophagy inhibitor (Mdivi) ameliorates pressure overload induced heart failure. PLoS One 7(3):e32388
141. Ferreira JCB, Campos JC, Qvit N, Qi X, Bozi LHM, Bechara LRG et al (2019) A selective inhibitor of mitofusin 1-betaIIPKC association improves heart failure outcome in rats. Nat Commun 10(1):329
142. Campos JC, Queliconi BB, Bozi LHM, Bechara LRG, Dourado PMM, Andres AM et al (2017) Exercise reestablishes autophagic flux and mitochondrial quality control in heart failure. Autophagy 13(8):1304–1317
143. Pisano A, Cerbelli B, Perli E, Pelullo M, Bargelli V, Preziuso C et al (2016) Impaired mitochondrial biogenesis is a common feature to myocardial hypertrophy and end-stage ischemic heart failure. Cardiovasc Pathol 25(2):103–112
144. Arany Z, He H, Lin J, Hoyer K, Handschin C, Toka O et al (2005) Transcriptional coactivator PGC-1 alpha controls the energy state and contractile function of cardiac muscle. Cell Metab 1(4):259–271

145. Herzig S, Shaw RJ (2018) AMPK: guardian of metabolism and mitochondrial homeostasis. Nat Rev Mol Cell Biol 19(2):121–135
146. Beauloye C, Bertrand L, Horman S, Hue L (2011) AMPK activation, a preventive therapeutic target in the transition from cardiac injury to heart failure. Cardiovasc Res 90(2):224–233
147. Han TS, Lean ME (2016) A clinical perspective of obesity, metabolic syndrome and cardiovascular disease. JRSM Cardiovasc Dis 5:2048004016633371
148. Bhatti JS, Bhatti GK, Reddy PH (2017) Mitochondrial dysfunction and oxidative stress in metabolic disorders—A step towards mitochondria based therapeutic strategies. Biochim Biophys Acta Mol Basis Dis 1863(5):1066–1077
149. Mootha VK, Lindgren CM, Eriksson KF, Subramanian A, Sihag S, Lehar J et al (2003) PGC-1alpha-responsive genes involved in oxidative phosphorylation are coordinately downregulated in human diabetes. Nat Genet 34(3):267–273
150. Heinonen S, Buzkova J, Muniandy M, Kaksonen R, Ollikainen M, Ismail K et al (2015) Impaired mitochondrial biogenesis in adipose tissue in acquired obesity. Diabetes 64(9):3135–3145
151. Kleiner S, Mepani RJ, Laznik D, Ye L, Jurczak MJ, Jornayvaz FR et al (2012) Development of insulin resistance in mice lacking PGC-1alpha in adipose tissues. Proc Natl Acad Sci U S A 109(24):9635–9640
152. Foretz M, Guigas B, Bertrand L, Pollak M, Viollet B (2014) Metformin: from mechanisms of action to therapies. Cell Metab 20(6):953–966
153. Zhou G, Myers R, Li Y, Chen Y, Shen X, Fenyk-Melody J et al (2001) Role of AMP-activated protein kinase in mechanism of metformin action. J Clin Invest 108(8):1167–1174
154. Bogacka I, Xie H, Bray GA, Smith SR (2005) Pioglitazone induces mitochondrial biogenesis in human subcutaneous adipose tissue in vivo. Diabetes 54(5):1392–1399
155. Diamant M, Heine RJ (2003) Thiazolidinediones in type 2 diabetes mellitus: current clinical evidence. Drugs 63(13):1373–1405
156. Bach D, Naon D, Pich S, Soriano FX, Vega N, Rieusset J et al (2005) Expression of Mfn2, the charcot-marie-tooth neuropathy type 2A gene, in human skeletal muscle: effects of type 2 diabetes, obesity, weight loss, and the regulatory role of tumor necrosis factor alpha and interleukin-6. Diabetes 54(9):2685–2693
157. Bach D, Pich S, Soriano FX, Vega N, Baumgartner B, Oriola J, et al (2003) Mitofusin-2 determines mitochondrial network architecture and mitochondrial metabolism. A novel regulatory mechanism altered in obesity. J Biol Chem 278(19):17190–171907
158. Wai T, Langer T (2016) Mitochondrial dynamics and metabolic regulation. Trends Endocrinol Metab 27(2):105–117
159. Lin MT, Beal MF (2006) Mitochondrial dysfunction and oxidative stress in neurodegenerative diseases. Nature 443(7113):787–795
160. Johri A, Beal MF (2012) Mitochondrial dysfunction in neurodegenerative diseases. J Pharmacol Exp Ther 342(3):619–630
161. Wang X, Su B, Lee HG, Li X, Perry G, Smith MA et al (2009) Impaired balance of mitochondrial fission and fusion in Alzheimer's disease. J Neurosci 29(28):9090–9103
162. Burte F, Carelli V, Chinnery PF, Yu-Wai-Man P (2015) Disturbed mitochondrial dynamics and neurodegenerative disorders. Nat Rev Neurol 11(1):11–24
163. Cho DH, Nakamura T, Fang J, Cieplak P, Godzik A, Gu Z et al (2009) S-nitrosylation of Drp1 mediates beta-amyloid-related mitochondrial fission and neuronal injury. Science 324(5923):102–105
164. Guo X, Disatnik MH, Monbureau M, Shamloo M, Mochly-Rosen D, Qi X (2013) Inhibition of mitochondrial fragmentation diminishes Huntington's disease-associated neurodegeneration. J Clin Invest 123(12):5371–5388
165. Rappold PM, Cui M, Grima JC, Fan RZ, de Mesy-Bentley KL, Chen L et al (2014) Drp1 inhibition attenuates neurotoxicity and dopamine release deficits in vivo. Nat Commun 5:5244
166. Joshi AU, Saw NL, Vogel H, Cunnigham AD, Shamloo M, Mochly-Rosen D (2018) Inhibition of Drp1/Fis1 interaction slows progression of amyotrophic lateral sclerosis. EMBO Molecul Med 10(3)

167. Waterham HR, Koster J, van Roermund CW, Mooyer PA, Wanders RJ, Leonard JV (2007) A lethal defect of mitochondrial and peroxisomal fission. N Engl J Med 356(17):1736–1741
168. Vanstone JR, Smith AM, McBride S, Naas T, Holcik M, Antoun G et al (2016) DNM1L-related mitochondrial fission defect presenting as refractory epilepsy. Eur J Hum Genet 24(7):1084–1088
169. Coskun PE, Beal MF, Wallace DC (2004) Alzheimer's brains harbor somatic mtDNA control-region mutations that suppress mitochondrial transcription and replication. Proc Natl Acad Sci U S A 101(29):10726–10731
170. Jiang H, Kang SU, Zhang S, Karuppagounder S, Xu J, Lee YK, et al (2016) Adult conditional knockout of PGC-1alpha leads to loss of dopamine neurons. eNeuro 3(4)
171. St-Pierre J, Drori S, Uldry M, Silvaggi JM, Rhee J, Jager S et al (2006) Suppression of reactive oxygen species and neurodegeneration by the PGC-1 transcriptional coactivators. Cell 127(2):397–408
172. Craven L, Alston CL, Taylor RW, Turnbull DM (2017) Recent advances in mitochondrial disease. Annu Rev Genomics Hum Genet 18:257–275
173. Ast T, Meisel JD, Patra S, Wang H, Grange RMH, Kim SH, et al (2019) hypoxia rescues frataxin loss by restoring iron sulfur cluster biogenesis. Cell 177(6):1507–1521 e16
174. Viscomi C, Bottani E, Civiletto G, Cerutti R, Moggio M, Fagiolari G et al (2011) In vivo correction of COX deficiency by activation of the AMPK/PGC-1alpha axis. Cell Metab 14(1):80–90
175. Quintana A, Kruse SE, Kapur RP, Sanz E, Palmiter RD (2010) Complex I deficiency due to loss of Ndufs4 in the brain results in progressive encephalopathy resembling Leigh syndrome. Proc Natl Acad Sci U S A 107(24):10996–11001
176. Khan NA, Auranen M, Paetau I, Pirinen E, Euro L, Forsstrom S et al (2014) Effective treatment of mitochondrial myopathy by nicotinamide riboside, a vitamin B3. EMBO Mol Med 6(6):721–731
177. Delettre C, Lenaers G, Griffoin JM, Gigarel N, Lorenzo C, Belenguer P et al (2000) Nuclear gene OPA1, encoding a mitochondrial dynamin-related protein, is mutated in dominant optic atrophy. Nat Genet 26(2):207–210
178. Zuchner S, Mersiyanova IV, Muglia M, Bissar-Tadmouri N, Rochelle J, Dadali EL et al (2004) Mutations in the mitochondrial GTPase mitofusin 2 cause charcot-marie-tooth neuropathy type 2A. Nat Genet 36(5):449–451
179. Zuchner S, Noureddine M, Kennerson M, Verhoeven K, Claeys K, De Jonghe P et al (2005) Mutations in the pleckstrin homology domain of dynamin 2 cause dominant intermediate charcot-marie-tooth disease. Nat Genet 37(3):289–294
180. Sato M, Sato K (2011) Degradation of paternal mitochondria by fertilization-triggered autophagy in C. elegans embryos. Science 334(6059):1141–1144
181. Perdomini M, Belbellaa B, Monassier L, Reutenauer L, Messaddeq N, Cartier N et al (2014) Prevention and reversal of severe mitochondrial cardiomyopathy by gene therapy in a mouse model of Friedreich's ataxia. Nat Med 20(5):542–547
182. Jang YH, Lim KI (2018) Recent advances in mitochondria-targeted gene delivery. Molecules 23(9)
183. Gerard C, Xiao X, Filali M, Coulombe Z, Arsenault M, Couet J et al (2014) An AAV9 coding for frataxin clearly improved the symptoms and prolonged the life of Friedreich ataxia mouse models. Mol Ther Methods Clin Dev. 1:14044
184. Yu H, Koilkonda RD, Chou TH, Porciatti V, Ozdemir SS, Chiodo V et al (2012) Gene delivery to mitochondria by targeting modified adenoassociated virus suppresses Leber's hereditary optic neuropathy in a mouse model. Proc Natl Acad Sci U S A 109(20):E1238–E1247

Metabolic Pathways in Immune Cells Commitment and Fate

Renan Willian Alves, Eloisa Martins da Silva, Lorena Doretto-Silva, and Vinicius Andrade-Oliveira

1 Introduction

For years, our perception of the importance of metabolic pathways for immune cell differentiation and function went unnoticed. However, in recent years, a remarkable increase in scientific findings demonstrated that metabolic pathways are crucial in modulating immune cell activation, differentiation, and function. The immune cells are important components of the immune system that work cooperatively, and along with lymphoid organs, are responsible to mount a proper response against body invaders, such as bacteria, fungi, parasites, viruses, and maintaining host homeostasis [1]. These processes sequentially involve the innate and adaptive systems, composed of monocytes, dendritic cells (DCs), macrophage (MΦ), and B and T cells, respectively [2].

Macrophages are myeloid progenitor-derived immune cells that compose the first line of immune defense, classified as mononuclear phagocytes. They can be derived either from circulating monocytes or being established in the embryonary phase from the Yolk sac, also known as tissue-resident macrophages, being subsequently maintained in an independent manner of monocytes [3–5]. Macrophages also participate in the processing and presentation of antigens for T cells as well as the clearance of the tissue apoptotic cells playing an essential role in controlling the inflammatory response during health and disease state [6, 7]. Along with these functions, macrophages produce and secrete several pro-inflammatory cytokines, such as tumor necrosis factor (TNF), IL-1, IL-6, IL-8, and IL-12 [8, 9]. Due to its broad spectrum

R. W. Alves · L. Doretto-Silva · V. Andrade-Oliveira (✉)
Center for Human and Natural Sciences, Federal University of ABC, Santo Andre, SP, Brazil
e-mail: andrade.vinicius@ufabc.edu.br

E. M. da Silva
Paulista Medical School, Federal University of São Paulo, Sao Paulo, Brazil

N. O. S. Camara et al. (eds.), *Essential Aspects of Immunometabolism in Health and Disease*, https://doi.org/10.1007/978-3-030-86684-6_4

of functions and location within the tissue, macrophages are considered a heterogeneous population. Having said that, traditionally, macrophages have been classified into pro-inflammatory (M1) or anti-inflammatory (M2) macrophages, both with distinct functions in the initiation and resolution of inflammatory processes [10]. In response to extrinsic stimulation in vitro, IFN-γ-stimulated bone-marrow (BM)-derived macrophages can differentiate into M1 macrophages that promote inflammation, whereas IL-4-stimulated-BM-derived macrophages turn into M2 macrophages that restrain inflammation [11]. However, recent evidence has revealed that alongside the cytokine stimuli and antigen recognition, metabolic pathways are essential for modulating macrophage plasticity [12–15]. These metabolic changes are a useful resource used by the macrophages to produce sufficient energy to support the needs required to mount a proper host immune defense and we will give some highlights about the role of these metabolic pathways in macrophage polarization and function.

B and T cells are components of the adaptive immune system being the B cells responsible for humoral immune response and presenting antigens to T cells, while the T cell is involved in cell-mediated immunity and production of cytokines [16–18]. Both B and T cells originate from hematopoietic stem cells produced in the BM [19]. In the BM, B cells remain and undergo successive selection processes to ensure proper development [20]. After the differentiation stage, B cells migrate to finish up the maturation process in the spleen. Once mature, B cells reside either in the follicles of the spleen and in lymph nodes [21]. Upon activation, these cells differentiate and give rise to either plasma cells, which produce antibodies to specific antigens, or memory B cells [22]. While B cells initiate the maturation process in BM and finish in the spleen, T cells mature in the thymus, giving rise to either CD4+ T cells, also known as helper T cells, or CD8+ T cells, cytotoxic cells, [23–25]. Given the importance of cellular metabolism for cell survival and function, it is reasonable to think that the activation and function of immune cells are under great influence of how these cells deal with the nutrients surrounding the microenvironment during activation/differentiation. Indeed, the role of how metabolic pathways alter the differentiation and function of both lymphocytes has gained special attention [26–31].

Nowadays, there is a comprehension that the transition of immune cells from quiescence to activated and differentiated stages requires the consumption of several metabolites or the redirecting of some metabolic routes [32–34]. Moreover, it has become even more evident that the basic function of immune cells can be regulated by the non-canonical function of the metabolic enzymes, placing metabolic enzymes to a new level of complexity in the regulation of the immune cell differentiation, fate, and survival [35–38].

Metabolic pathways are a group of biochemical reactions occurring in all living cells and supply energy and substrate for cell maintenance. Glycolysis is the simplest process to generate cellular energy and takes place in the cytosol. This metabolic pathway involves the conversion of glucose into pyruvate, thus generating two ATP molecules per glucose without consuming oxygen [39, 40]. At the end of glycolysis, the newly synthesized molecules of pyruvate are oxidized into acetyl coenzyme A (Acetyl-CoA), which is the initial substrate for the tricarboxylic acid cycle (TCA) of

cellular respiration [41, 42]. Next, TCA uses Acetyl-CoA and other substrates generating the reduced form of electron carriers, NADH and $FADH_2$, also giving origin to several molecules that supply various biosynthetic processes [42]. In mitochondria, both NADH and $FADH_2$ transfer their electrons to O_2 by a series of electron carriers, a process called oxidative phosphorylation (OXPHOS), generating about 30 molecules of ATP for the cell [43]. Another metabolic pathway crucial for cell survival is the metabolism of fatty acids. Lipid metabolism involves two separate pathways that lead to either the synthesis or degradation of fatty acids that take place in the cytosol and within the mitochondria, respectively [44]. Fatty acid oxidation is a catabolic process that converts aliphatic compounds into units of Acetyl-CoA generating energy and substrate for TCA, whereas fatty acids synthesis creates polymeric molecules, such as triglycerides, hormones, phospholipids from monomers subunits [45–47]. In addition, amino acid metabolism is an alternative pathway that provides essential substrates for new protein synthesis which has a significant demand for proliferating cells, such as immune cells [48]. Recent evidence has demonstrated that amino acids are related to the activation and proliferation of B, T lymphocytes, and macrophages [49–52].

The contribution of metabolic molecules in controlling the activation and response of immune cells under homeostasis and during inflammatory disease remains to be fully uncovered. Here, we review the various metabolic states in immune cells and how they influence the immune cell's function, proliferation, activation, and differentiation.

2 Metabolic Pathway Impact on T Cell Differentiation and Function

T cell differentiation begins when the hematopoietic stem cell migrates BM to the thymus and starts its maturation. Double-negative (DN) cells in the BM have early stimulation from Notch1 receptor signaling which causes the appearance of thymic-independent T cell progenitors, blocking early B cell differentiation [53]. For this signaling, Notch promotes glucose uptake in pre-T cells and requires the PI3K-Akt pathway, important for cell growth and survival, to promote glucose metabolism [54]. Phosphatidylinositol 3-kinase (PI3K) has three classes and together they are responsible for regulating a variety of signaling pathways within the cells such as glucose homeostasis, cell metabolism, growth, proliferation, and survival, membrane traffic, and autophagy [55, 56].

Serine/threonine kinase (Akt) is an important PI3K pathway mediator [55] and in combination, PI3K/Akt maintaining activation and activity of glucose transporter 1 (Glut1) [57] which is essential in thymocytes for the beginning of the proliferative stages of the double negative cells [58]. Also, the PI3K/Akt signaling pathway engaged with the mammalian target of rapamycin (mTOR) involving many cellular functions and metabolism [59]. Interferences in PI3K signaling can directly interfere

in the T cell maturation process as PI3K subunits p110y is linked with the transition Double Negative to Double Positive and, in the positive selection p110y deficiency reduced CD4+/CD8+ T cell differentiation [60].

Naïve T cell survival requires IL-7 signaling and TCR interaction with self-antigen, the major histocompatibility complex (MHC) class I and II, that correspond to mature TCD8+ and TCD4+ respectively [61]. Interleukin 7 (IL-7) is an essential cytokine for naïve T cell survival and development [50] such as its absence or deficiency in IL-7 receptor is linked with patients with severe combined immunodeficiency [62]. Notch signaling induces an IL-7-driven proliferation of CD4+ single-positive cells and IL-7 can also influence Notch signaling increasing cell survival [63]. Additionally, IL-7 regulates glucose uptake depending on transcription 5 (STAT5) activity which promotes Akt activation that regulates Glut1 localization on the cell surface [64]. After thymic selection, T cells trafficking to a secondary lymphoid organ, where they can stimulate with cognate antigen and get activated. Activation of T cells requires different metabolic pathways to supply their energy needs, mainly glucose and glutamine for growth and proliferation [50].

In a second lymphoid organ, such as lymph node or spleen, naïve T cell is presented to a specific antigen, which triggers naïve T cells to undergo a clonal expansion and generate a considerable number of effector T cells, as well as produce cytokines, chemokines, and other effector molecules. To this happening, naïve T cell needs a considerable quantity of energy, so it reprograms its metabolism during the activation process to express more glucose transporters and initiate aerobic glycolysis [40].

T cells express glucose transporters from the Glut family, mainly Glut1, and it is completely dependent on the activation of intracellular pathways such as the mammalian target of rapamycin 1 (mTORC1), the oncogenic transcription factor c-Myc, and hypoxia-inducible factor 1α (HIF1α) to coordinate this process [40]. For the rapid glycolysis needed after activation, all these pathways aforementioned are also triggered upon the T cell receptor (TCR) and CD28 co-stimulatory pathways activation, which culminates with the stimulate Glut1 and Akt pathway-dependent Glut1 trafficking to the cell surface increasing the glucose uptake [65]. Myc function also plays an important role in other metabolic pathways induced upon naive T cell activation. Myc activation correlates with an increase not only of the aerobic glycolysis but also of the pentose phosphate pathway (PPP) and glutamine oxidation, suggesting that Myc is important to coordinate the induction of these pathways for T cell activation [50].

Indeed, resting and/or quiescent T cells oxidize glucose to pyruvate, together with lipids and amino acids, generating a large amount of ATP, and once T cells become activated, glycolysis and glutaminolysis increase while lipid oxidation decreases [66]. For not completely understood reason, T cells undergo activation chose to produce ATP from glucose metabolism through lactate production rather than by pyruvate oxidation into acetyl-CoA, even in the presence of oxygen, a phenomenon called the Warburg effect, first demonstrated in cancer cells [67]. It is assumed that this effect ends up supporting the T cell to generate molecules that are necessary to build organelles and other cellular structures to assure condition to T cell to proliferate. In addition, increased aerobic glycolysis in activated/effector T cells prevents the

accumulation of molecules from the glycolytic pathway, such as GAPDH, which is able the inhibit IFN-γ production, decreasing the effector function of the CD4+ T cell [68]. Moreover, aerobic glycolysis and consequently lactate production seems to increase the Acetyl-Coa pool in Th1 cells, which facilitate the histone acetylation of IFN-γ locus, linking aerobic glycolysis with Th1 cell differentiation through an epigenetic-dependent mechanism [69].

Like T cell development and maturation, metabolic programs support CD4+ T cell differentiation in Th subsets differentially and each one has a specific metabolic profile: Th1, Th2 and Th17 remain with aerobic glycolysis and have as a key regulator mTORC1, mTORC2 and mTORC1/HIF1α, respectively. Unlike them, regulatory T (Treg) cells maintain lipid oxidation and AMPK as a regulator [66].

Note that mTOR although has a cross-talk with the Myc pathway during glutaminolysis [50] as an immunosuppressive drug, it helps to promote the generation of FoxP3+ cells [71]. Despite the fact that mTORC1 deletion increases Treg numbers, the absence of mTORC1 specifically in Treg completely impaired its function [72]. In addition, pyruvate dehydrogenase (PDH), a metabolic enzyme that catalyzes pyruvate into acetyl-CoA beginning the TCA cycle [73], is a key bifurcation between CD4+ T cells to convert pyruvate to lactate or acetyl-CoA, being an important regulatory node for Th17 and Treg. PDH inhibitor, PDHK is differentially expressed in T cells subsets and have a selective regulator for Th17/Treg balance [74].

Each cell state requires different metabolic demands with different signaling pathways to provide the energy that the cell needs in their specific development moment. (Fig. 1—superior part) illustrates only glycolysis and oxidative phosphorylation, to

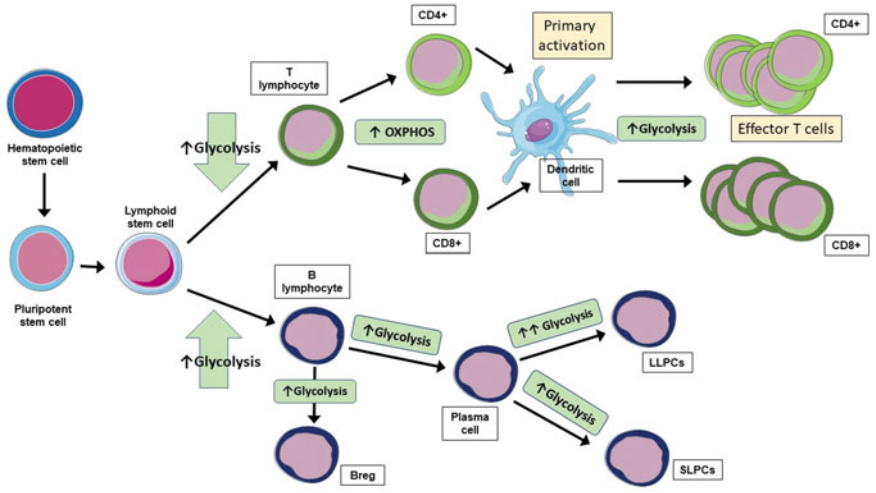


Fig. 1 Metabolic pathway influences on T and B cell differentiation. Lymphoid stem cells during differentiation suffer stimulations from cytokines and aerobic glycolysis to differentiate in T or B cells. Naive T cells use OXPHOS for their maturation process, and sometimes glycolysis during proliferation. Naive B cells use glycolysis for their maturation process

show how complex it is for the cell to choose a better metabolic process to generate ATP during their differentiation.

3 Effector Versus Memory Cells

T cells are considered naïve before their contact with its cognate antigen in a secondary lymphoid organ. Then, the cells become effectors and as the antigen is progressively decaying because of the effective immune response, antigen-specific effector T cells number decline as well and some of them will give rise to memory T cells [75]. There are three subtypes of memory cells, central memory (TCMs) and the effector memory (TEMs) and tissue-resident memory (T_{RMs}), characterized and differentiated according to specific markers and functions [76]. TCMs support reactive memory, usually do not have an effector function, but proliferate and differentiate in effectors after re-encountering with antigen, are CD45R0+, IL-2 producer, express CCR7 and CD62L that are necessary for migration to T-cell areas of the secondary lymphoid, less dependent of co-stimulation, compared to naïve T cells [77, 78]. The TEMs are characterized by the low expression of CCR7, CD62L, and high expression of CD44, produce some effector cytokines, such as IFN-γ or IL4 and IL5 after TCR re-stimulation and TEMs CD8+ migrate to the inflamed tissue and have immediate effector function [76, 77].

At homeostasis, the naive T cells have relatively low energetic demands and generate energy through OXPHOS [79], while TEMs rely less on OXPHOS, mainly using aerobic glycolysis [76, 80]. As mentioned before, after an antigen encounter, naive cells begin to divert glucose from mitochondria and induce aerobic glucose through enzyme pyruvate dehydrogenase kinase 1 (PDK1), supporting the needs of proliferating effector cells [30]. In addition, some transcription factors and pathways, Akt and mTOR, c-MYC and HIF-α, respectively, induce transcription of metabolic genes for the correct development of these cells [76]. Indicating that aerobic glycolysis is the most important metabolic pathway for the development of effector T cells.

The process of passage from effector T cell to memory T cells is dependent on a metabolic remodeling for the cell to acquire this phenotype [76]. There is a decrease in metabolic pathway activities necessary for effector T-cell development, such as mTOR and aerobic glycolysis, governed by signaling molecules such as IL-7, IL-15 and IL-21 [71, 81]. For the maintenance and regulation of CD8+ memory cells, an increase in the mitochondrial fatty acid oxidation and spare respiratory capacity [80, 82] increases the development of CD8+ memory cells ($CD8^+T_M$) [77]. This FAO improves T cell survival and development of $CD8^+T_M$ cells, as well as IL-2 support OXPHOS for effector T cells (T_E) development [82, 83]. Thus, metabolic modulation biased to mitochondrial metabolism, mainly in FAO, are fundamental for the development of T_M (Fig. 2).

In addition to these two well-described subtypes, TCMs and TEMs, more recently TRM gained to focus on research on infection and metabolism [84, 85]. Unlike TEMs

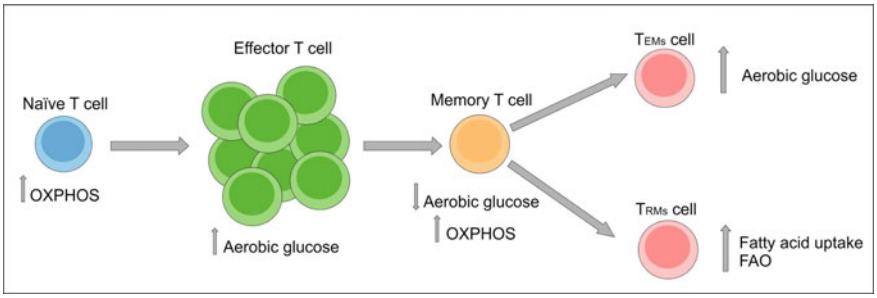


Fig. 2 Metabolic profile effector versus memory T cell. The differentiation of effector T cells from naive T cells has specific metabolic alterations to perform their functions. Effector T cells have an increase in aerobic glucose to maintain their effector activity and increase proliferation. When they become memory T cells they do not require a large energy demand, decreasing aerobic glucose and increasing OXPHOS activity. After re-exposure to the antigen, the effector memory and tissue-resident memory T cells increase aerobic glucose and FAO, respectively

and TCMs, TRM does not circulate through the blood, remaining in the tissues for long periods [86, 87]. The TRMs triggers an immediate immune response after re-exposure to the antigen, functioning as the first line of defense in response to a specific antigen, as a bridge between the adaptive and innate immune system [87]. In regard to the metabolism of TRMs cells, it is known that it is specific to each tissue [85, 88]. For instance, TRM CD8+ cell in the skin adapts to use lipid metabolism by using exogenous free fatty acid (FFAs) through the high expression of molecules related to lipid uptake, Fatty acid-binding protein 4 (FABP4) and Fatty acid-binding protein 5 (FABP5), in response to immunization by viruses [85]. Similarly to the skin, in adipose tissue, TRM cells showed high rates of lipid uptake and mitochondrial respiration, with high proliferation, suggesting that adipose tissue is an ideal metabolic environment for memory T-cell longevity [88]. In addition, intestinal CD8+ TRM also has increased FFA pathways and cholesterol synthesis that can be made available for FAO [89]. In the context of tumors, dependence on fatty acids of TRM may be favorable, since tumor cells are highly glycolytic and make the environment hypoglycemic, but it is oxygen dependent and tumors typically have a hypoxic environment [90]. In gastric adenocarcinoma, the presence of TRM is a good prognosis [91], where TRM $CD103^{high}$ cells have higher lipid uptake and lower glycolytic capacity [92]. A recent study showed that TRM $CD103^{high}$ cells supplemented with FFAs increased cytokine production wheres the inhibition of fatty acid oxidation impairs TRM $CD103^{high}$ survival and PD-L1 blockade restored the number of TRM cells by increasing the expression of molecules related to lipid uptake FABP4 and FABP5 [92]. This data suggests the metabolic reprogramming of TRM cells is a potential therapeutic target in gastric neoplasia.

In short, naïve T cells have reduced mitochondrial activity and glucose uptake generating ATP mostly from OXPHOS [93]. Once activated, these cells exit quiescent states passing through a cellular metabolism reprogramming, such as an increase in glucose, glutamine uptake, and fatty acids synthesis [50, 94, 95]. Memory T cells, in

turn, rely basically on glucose and fatty acids oxidation to support their metabolism [96, 97]. Moreover, investigating how immune and metabolic signals regulate T cell fate could unwrap new roles in T cell dysfunctions in infectious, autoimmune disease, and cancer.

4 B Cell Metabolism

B cells are members of the adaptive immunity, and their development occurs in the bone marrow (BM) from hematopoietic cells [98]. The entire repertoire of B-cell antibody production occurs through a gene rearrangement, known as VDJ [99]. B cells spend most of their quiescent time recirculating in lymph and blood and only after exposure to an antigen they undergo clonal expansion and ultimately differentiate into short-lived plasma cells, memory, or long-lived antibody-secreting plasma cells [100]. One of the factors that can compromise the development and maturation of B cells is cellular metabolism [101]. During the development of B cells, they suffer different energy demands, according to their size and need for proliferation. For instance, B-cell precursors increase glucose uptake, oxygen consumption, and glycolysis rate [102]. Moreover, the highest glucose uptake is observed in large pre-B cells, while small pre-B cells exhibit lower levels of lactate secretion and oxygen consumption [103]. This suggests that B cells depend on glucose for their development and in fact, deficient mice of the glucose transporter GLUT1 in B cells have a reduced number of mature B cells [104]. Inhibiting glucose at various stages of the B cell development showed that early pre-B cells are highly dependent on the glycolytic pathway [101], evidencing the importance of metabolic reprogramming of B cells during its development phases.

Comparing naive B-cell metabolism with activated B cells, we have an increase in glucose uptake, oxygen consumption, and lactate secretion in B cells upon activation [104]. Some metabolic interventions, such as treatment with 2-deoxyglucose (2-DG), a derivative of glucose that is not metabolized by the cell, which inhibits glycolysis [105] and mitochondrial respiration [106], have been shown to reduce b-cell proliferation and survival [105, 106]. The regulation of some cytokine signaling in B cells may alter the behavior of B-cell metabolism [107]. For example, IL-4 cytokine increases glucose and glucose transport in B cells, and blocking glycolysis with 2-DG leads to decreased survival of B cells [108]. One pathway involved with B cell metabolism is mTOR and when its activity is impaired, glycolysis and oxygen consumption decrease, demonstrating the importance of this pathway for adequate metabolic activity and consequently optimal cellular development [109]. In addition, glucose uptake seems to depend on different molecules [110]. B cells with high expression of oncogene MYC depend more heavily on OXPHOS [111], also, deficiency of an essential regulator of B-cell survival (TRAF3), promotes increased glucose uptake, with increased anaerobic glycolysis and mitochondrial [112]. Moreover, it is known that memory B cells can differentiate into plasmablasts [113] and

that the activation of mTORC1 and the increase in glycolysis results in this differentiation, thus, metabolic reprogramming compromises memory B-cell differentiation in plasmablasts [114]. Furthermore, homocysteine (HCY) that activates B-cell proliferation and antibody secretion, increases B-cell metabolism (OXPHOS and glycolysis) dependent on PKM2 [115]. These data suggest that metabolic reprogramming impacts the survival, maturation, and production of antibodies in B cells [105, 106, 115].

Regarding plasma cells (PCs) they have their origin in secondary lymphoid organs, spleen, and lymph nodes [116]. After contact with antigen through its BCR surface receptor, they undergo clonal expansion and differ into two long-lived plasma cells (LLPCs) and short-lived (SLPCs) subpopulations, with the former typically formed in GC [117, 118]. After re-exposure, SLPCs are found mainly in the spleen and lymph nodes and LLPCs in BM and some gut-associated lymphoid tissue (GALT) [118]. Peyer's Patches (PP) are the predominant GALT in the small intestine, and the predominant B-cell population is B naive [119]. Differentiated lamina propria (LP) IgA + PCs cells preferentially use glycolysis compared to naive B cell [120], evidencing glucose importance in plasmocyte differentiation and antibody production. This data also corroborates the inhibition of GLUT1 in B cells decreasing antibody production [121]. Moreover, IgA-producing PCs have a vitamin B dependence for differentiation [122]. While the naive B cells of PP generate ATP mainly through TCA using amino acids and fatty acids, PCs in LP use TCA through glycolysis [119]. Thus, vitamin B deficiency impairs TCA and impairs the survival of naive B cells, while LP PCs can survive through glycolysis [120]. About plasma cell metabolism, LLPCs import more glucose than short-lived plasma cells (SLPCs) in homeostasis and, under metabolic stress, LLPCs, but not SLPCs, divert glucose to form mitochondrial pyruvate [123]. Recently, a regulator of glucose metabolism, the enzyme ATP ENPP1, has been demonstrated as essential for the development and survival of LLPCs in mice [124], also, LLPCs have a higher respiratory capacity than SLPCs, suggesting differences in the respiratory capacity that may be associated with survival [123, 124].

Another subpopulation of B lymphocytes is the regulatory B-cell (Breg), identified by markers CD1d high and CD5+ [125]. Although much less studied than other types of regulatory immune cells, Breg has shown an essential role in various situations of homeostasis and inflammation [126]. Even as other regulatory immune cells, Breg produces IL-10, Transforming growth factor-beta (TGF-β), and IL-35, all with known regulation functions [127]. In addition, Breg can suppress pro-inflammatory leukocytes, such as TNFa-producing monocytes and DCs, and also induce differentiation of Treg lymphocytes [126]. Xianyi Meng [128], showed the high glycolytic potential of Bregs and, HIF-α-deficient Breg has a lower cell expansion, demonstrating the need for HIF-α expression for expansion of these cells and their glycolytic profile [128] a role observed for other immune cell population [129].

Non-Hodgkin lymphomas (NHLs) are characterized by the presence of large B cells, all with deregulated mTORC1 activity [130]. The large B cells are neoplastic cells found in diffuse large B cell lymphoma (DLBCL), a subtype of NHLs. In vitro metabolic characterization of BCR-DLBCL (large B cell) demonstrates its highly

glycolytic profile and a lower number of mitochondrial proteins [131]. Metabolism-related proteins have been associated with the severity of DLBCL, the presence of proteins related to glycolytic activity, such as monocarboxylate transporter 1 (MCT1) and monocarboxylate transporter 4 (MCT4) are associated with a more aggressive form of the disease, as well as the glucose transporter GLUT1 [130, 132]. However, genetic analysis of primary DLBCL showed the presence of genes involved in mitochondrial metabolism in 30% of patients [133], demonstrating heterogeneity in diffuse large B cell lymphoma and an important role of the metabolism of these cells in their development [130, 133].

To summarize, many studies reported that naïve B cells have a low level of metabolic activity. When these cells are activated is observed an increase in glucose uptake, oxygen consumption and lactate secretion which is related to glycolysis and OXPHOS pathways, reflecting a change in energetic and biosynthesis demands [104]. In contrast, GC B cells consume high levels of glucose in comparison to naïve B cells, also being more sensible for glycolysis inhibition suggesting that this pathway is important for the survival and proliferation of GC B cells [105, 134] (Fig. 1—inferior part). Future studies should investigate the interaction of stimuli from the microenvironment, such as cytokines and intracellular signaling pathways and metabolic regulation on immune cells in the context of metabolic disease and dysfunctions.

5 Macrophages M1/M2

Macrophages are cellular components of the innate immune system that reside in the most diverse tissues and play an essential role in several immune response processes such as maintaining homeostasis and regulating inflammatory processes [135]. Phenotypes of macrophages are determined by environmental stimuli and can be divided into two subgroups, depending mostly on the Th1/Th2 stimulation [136]. Inflammatory (M1) macrophages are activated by (IFN-γ) and lipopolysaccharide (LPS) stimulation and are associated with inflammatory response [137–139]. In contrast, alternatively-activated or (M2) macrophages are activated by IL-4 and IL-10 stimuli and are involved in anti-inflammatory response and tissue remodeling [137–140]. In fact, it is now established that the M2 is divided into four subtypes, specifically, M2a, M2b, M2c, and M2d distinguished on their inducing agent and gene expression markers [141–143]. It has been demonstrated that cellular metabolism can modulate not only the differentiation of monocytes into macrophages but also regulates the activation of macrophages and other immune cells such as DCs [144–147]. In addition, there is also data supporting the influence of the metabolic pathways in macrophage polarization [12, 15, 148, 149]. For instance, LPS-stimulated macrophages have an increase in glycolysis while IL-4-stimulated macrophages support an increase in OXPHOS. So, these metabolic changes are crucial for controlling inflammation and tissue repair responses [150, 151].

Metabolic reprogramming in macrophage phenotype was analyzed by in vitro polarization studies [152]. Inflammatory macrophages activation depends on aerobic glycolysis as the main source of ATP for the production of reactive oxygen species (ROS), a useful component for the host defense against microbes [153]. In contrast, regulatory macrophage activation requires FAO to boost mitochondrial oxidative phosphorylation (OXPHOS) and generate ATP [154]. Besides the impact of glycolysis in the macrophages activation, other key metabolic cascades such as the pentose phosphate pathway, oxidative phosphorylation, and amino acid metabolism can modulate this process [155, 156] and we are covering the importance of these pathways below. These studies prove that macrophage polarization can be regulated by distinct metabolites states in cellular metabolism.

It has been well established for decades that the upregulation of glycolysis is necessary for the macrophages activation. The first studies in the field of macrophages immunometabolism appeared in the 1970s. Harold et al. concluded that M1 macrophages exhibited an increase in glycolysis rate accompanied by a reduction in oxygen consumption [157, 158]. Pavlou et al. analyzed the administration of 2-DG and observed that blocking the glycolysis can inhibit M1 phenotype including secretion of the pro-inflammatory cytokine, opsonin-mediated phagocytosis, and production of ROS [158, 159]. The role of glycolysis in M2 macrophages is still unclear but some studies observed that blocking the glycolysis with 2-DG reduced the expression of the M2 differentiation markers [160–162]. In spite of, recent data suggested that glycolysis is not involved in M2 polarization as long as OXPHOS remains effective, indicating that in M2 macrophages activation OXPHOS can supply the metabolic activity in absence of glycolysis [163].

The lactate resulting from pyruvate reduction also affects the function of macrophages. Colegio et al. showed that the lactate secreted by tumor cells directs the M2 polarization toward Tumor-associated macrophages (TAM) and this phenotype helps tumor growth [165]. More recently, it was described as a non-metabolic role of lactate in epigenetic control in M1 macrophage polarization. In the late stages of M1 polarization, lactate can be used as a substrate for the lactylation of histone lysine residues which culminates in anti-inflammatory gene transcription toward an M2-like phenotype, promoting homeostasis [166].

After glycolysis, pyruvate is generated and it is converted into Acetyl-Coa, an important substrate to initiate the Tricarboxylic Acid (TCA) cycle. In addition to the changes observed in the glycolytic flux observed in macrophages activation, remarkable alterations were also described in TCA. Regulatory macrophages (M2) exhibit an intact and functional TCA cycle cooperatively with OXPHOS to generate sufficient ATP for the glycosylation of lectin and mannose receptors, which are highly expressed on M2 macrophages [167, 168]. On the other hand, the glycolytic metabolism observed in M1 macrophages is accompanied by metabolic changes in the TCA cycle. This disruption in the TCA cycle occurs mostly when macrophages are stimulated with LPS or other inflammatory mediators [13, 167] resulting in the accumulation of some metabolites such as citrate, itaconate, and succinate [148, 169]. M1 macrophages exhibit increased levels of citrate which alters the gene expression and plays an essential role in bearing the inflammatory response [169]. Briefly, citrate

is converted to isocitrate and subsequently to α-ketoglutarate due to the action of the isocitrate dehydrogenase (IDH) enzyme. Besides, citrate can be exported from the mitochondrial matrix to the cytosol through the mitochondrial citrate carrier (CIC). Jha et al. reported the downregulation of IDH [167] and Infantinno et al. analyzed the upregulation of CIC mRNA levels in LPS-stimulated macrophages, both of these phenomena causing direct citrate accumulation [170, 171]. Once in the cytosol, citrate appears to be critical for NO, ROS, and prostaglandin E2 (PGE2) production [170, 172]. The itaconate is the product of the citrate-derived cis-aconitate conversion through the activity of the Immune-responsive gene 1 protein (IRG1), as described by Michelucci et al. and is a metabolite with increased levels in activated macrophages [173]. Upon stimulation with LPS, some data reported the upregulation of IRG1 in cell lines and murine M1 macrophages [174]. Itaconate was found to inhibit succinate dehydrogenase (SDH), the enzyme responsible to convert succinate to fumarate, leading to the accumulation of this metabolite in activated macrophages [175, 176]. This increase in succinate levels leads to a decrease in mitochondrial respiration, ROS production, pro-inflammatory cytokine release, and inflammasome assembly during macrophage activation [177]. Moreover, Lampropoulou et al. using treatment with exogenous itaconate, concluded that itaconate acts as an anti-inflammatory by inhibiting SDH action, thus affecting the production of pro-inflammatory cytokines in macrophages in vitro and in vivo [177]. Also, itaconate prevents the degradation of the anti-inflammatory transcription factor nuclear factor erythroid 2-related factor 2 (NRF2) which accumulates and translocates to the nucleus and stimulates the transcriptional anti-oxidant and anti-inflammatory response genes [178]. In concern of M2 macrophages, the role of itaconate is not clearly understood. Despite IRG1 importance in impairing M1 macrophage response, O'Neil et al. observed that IRG1 expression is specific for M1 macrophages and has the opposite role in IL-4-dependent M2 macrophage polarization. When IRG1 is overexpressed in murine M2 macrophages or exogenous treatment with a derivative of itaconate, it inhibits M2 gene expression and blocks Stat6 and AKT pathway in IL-4-stimulated macrophages, also reducing OXPHOS in these cells. The authors suggested that itaconate inhibits phosphorylation of Janus Kinase 1 dampening the M2 activation. On the other hand, a microRNA molecule specific for M2 macrophages, miR-378a, suppresses the production of itaconate by these cells. Additionally, mR93 decreases the levels of IRG1 and itaconate in consequence of the reduction of interferon regulatory factor 9 (IRF9) which leads to an M2 macrophage polarization. These findings indicate a possible mechanism in which different concentrations of itaconate can modulate macrophage polarization.

Succinate is an intermediate metabolite of TCA and its role in the macrophages' metabolism was defined recently. Tannahill et al. described that the succinate levels were increased in LPS-stimulated macrophages and induced the activation and upregulation of pro-inflammatory genes, specifically, the IL-1β gene [176]. Beyond the succinate importance in the glycolysis pathway, another finding related to succinate was the identification of its function as a signaling substrate at an extracellular level. During inflammation, activated M1 macrophages release succinate which accumulates in the extracellular milieu [179]. Outside the cell, succinate can bind to the

succinate receptor GPR91, a G-protein coupled receptor sensor for extracellular succinate and can be expressed in many cell types [180]. Intriguingly, macrophages express GPR91, and in response to inflammatory stimuli occurs the transduction of pro-inflammatory phenotype and IL-1β production [181, 182]. This mechanism activated by succinate boosts the inflammation in an autocrine and paracrine manner to endure and intensify the inflammatory response [182, 183]. These observations demonstrate that succinate oxidation via SDH activity regulates ROS production [184]. Also, it was described that in LPS-stimulated macrophages the production of ROS can lead to DNA damage. In this situation, NAD^+ is consumed in a manner to activate the enzymes poly (ADP-ribose) and in consequence of this NAD^+ unbalance, the NAD^+ salvage pathway supports the NAD^+ flux by recycling nicotinamide into new NAD [185]. Altogether, these findings explain the reason why the NAD^+ salvage pathway is enhanced on inflammatory macrophages.

Following, the TCA cycle provides the necessary substrate reactions for the complexes of the electron transport chain (ETC) to generate ATP through proton flow and ATP synthase activity. The metabolic difference between M1 and M2 cells affects the way ATP is synthesized. Classically activated macrophages (M1) depend on glycolysis to produce most of their ATP while alternatively-activated macrophages (M2) utilize OXPHOS for the same purpose. Consequently, M2 macrophages sustain the electron forward ETC to generate ATP [167]. Vats et al. demonstrated the upregulation of OXPHOS diminishing the production of pro-inflammatory cytokines IL-4-treated macrophages [150]. Additionally, IL-25 and IL-10 were found to stimulate M2 polarization and increase macrophage mitochondrial respiratory activity [186, 187]. Nevertheless, in LPS-stimulated M1 macrophages, OXPHOS is diminished and, in consequence, dysregulated ETC leads to a leak of electrons that in presence of oxygen produces ROS [153, 188]. This mechanism is used by pro-inflammatory macrophages to adjust the ETC to reinforce the ROS production. Also, Van den Bossche [14] shows that nitric oxide (NO) produced by M1 macrophages inhibits OXPHOS and prevents the repolarization of M1 to M2 phenotype, and when NO production is inhibited occurs a notable rise in repolarization.

Pentose phosphate pathway (PPP) is also altered in macrophages differentiation, predominantly during the activation of M1 macrophages to supply the ATP demand [167]. This pathway is an alternative route for the oxidation of glucose-6P, in the cytosol, without generating ATP, instead of providing pentose and 5-ribose phosphate for nucleic acid synthesis but also donating electrons to the reduction of NADP+ to NADPH. In fact, the PPP is upregulated in M1 macrophages [167, 176] because NADPH is the major substrate for NADPH oxidase which catalyzes the ROS production that is essential for pathogens control [189]. Baardman et al. has shown that the knockdown of 6-phosphogluconate dehydrogenase (PGD), the enzyme responsible to convert 6-phosphogluconate into ribulose 5-P, produces an impaired pro-inflammatory response in macrophages [190]. Concerning M2 macrophages, Haschemi et al. suggest that occurs a suppression in PPP due to the downregulation of sedoheptulose kinase (CARKL), a carbohydrate kinase-like protein that controls the PPP and in this case results in a defective inflammatory response [191]

Furthermore, M1 and M2 macrophages are also distinguished by specific pathways in lipid metabolism to modulate their response. In general, lipid synthesis is related to the pro-inflammatory macrophage functions. Lipid synthesis is an essential pathway to provide the substrates necessary for membrane remodeling and the generation of inflammatory mediators, such as ROS and NOS, in M1 macrophages [172, 192]. In these cells, glycolysis is upregulated and Feingold et al. showed that the carbons arising from glucose-derived were, alternatively, incorporated in fatty acid molecules [193]. In consequence, there are also data supporting a rise in citrate and lipid levels in LPS-stimulated macrophages [167, 176]. In contrast, fatty acid degradation (beta-oxidation) is associated with M2 macrophages, which take up the free fatty acid to fuel OXPHOS and to produce energy via FAO [194]. Indeed, FAO is extremely important for M2 polarization. Malandrino et al. using etomoxir, a mitochondrial carnitine palmitoyl-transferase 1 (mCPT1) inhibitor to block FAO, demonstrated that in this case the M2 activation in IL4-stimulated macrophages is blocked [195]. When the authors expressed mCPT1 exogenously in macrophages the induction of pro-inflammatory phenotype was inhibited. Despite these results, CPT1 expressed is increased in inflammatory macrophages converting FA that, in sequence, are stored in lipid droplets (LD) [196]. In LPS-stimulated macrophages, LD is related to inflammatory functions, such as IL-1β, IL-6 and PGE2 production, and phagocytic capacity [197]. When LD development is diminished through the inhibition of triacylglycerol synthesis the proinflammatory functions described above are dampened [197], demonstrating a physiological role for LD in inflammatory macrophages.

Amino acids metabolism is an important component of many cell processes, including organizing an appropriate immune response. Early in the 1980s, Newsholme et al. [198, 199] dedicated to studying the consumption of amino acids upon macrophages activation. So macrophages can adapt to changes in nutrient sources and utilize amino acids catabolism, mainly, arginine, glutamine, and tryptophan to maintain their immune response and regulate activation [148, 200]. Arginine, for example, is the substrate for NO production through the activity of inducible nitric oxide synthase (NOS). Under pro-inflammatory stimuli, like LPS, TNF-α, or IFN-γ iNOS is overexpressed directing the arginine toward NO production [169, 200]. Furthermore, NO intensifies the macrophages' ability in killing intracellular bacteria during inflammation. Therefore, M1 macrophages utilize arginine and iNOS to generate pro-inflammatory NO. Van den Bossche also observed that NO plays a role in restraining M1 to M2 repolarization and once the iNOS is blocked, M1 is capable of repolarizing into M2 when stimulated with IL-4 [14]. In alternatively-activated macrophages, arginine is metabolized by enzyme arginase (ARG1) which is overexpressed in this phenotype [201]. This process generates ornithine and then is converted into proline and polyamines, important substrates for wound healing and cell proliferation, respectively [148, 169, 200]. Recently, Hardbower et al. demonstrated that the ornithine conversion limits M1 activation through histone modifications [202]. Also, ARG1 in macrophages plays a key role to activate anti-inflammatory phenotype and control T cell activation and proliferation [181, 203]. Glutamine can also modulate macrophage polarization and is the most available amino acid in the plasma [204]. During macrophages activation,

glutamine metabolism can modulate both M1 and M2 phenotype. The internalization and metabolism of the glutamine into the TCA cycle is the principal route to succinate synthesis in M1 macrophages [205] activating the transcription of glycolytic genes maintaining the glycolytic metabolism and pro-inflammatory response in these cells [176, 206]. Thus, not surprisingly, there is cooperation among metabolic pathways in promoting macrophage function. In M2 macrophages, glutamine directs M2 polarization by generating α-ketoglutarate from glutaminolysis. This metabolite intensifies M2 OXPHOS and FAO, stimulates M2 phenotype by epigenetic reprogramming [207], inhibits the transcription of glycolytic genes, and induces the glycosylation of lectin or mannose receptors which is expressed in M2 macrophages [167]. Liu et al. demonstrated that glutamine deprivation impaired the expression of M2 markers even after the activation with IL-4 [207]. Alternatively, M2 macrophages can induce the glutamine synthesis from glutamate using the glutamine synthetase (GS) enzyme [169]. The expression of GS is highly detectable in M2 macrophages, mainly, after the activation with IL-10, and while in M1 macrophages GS expression is hardly measurable, as described by Palmieri et al. [208]. Tryptophan metabolism is mediated almost by the enzyme indoleamine 2,3-dioxygenase (IDO) responsible for converting tryptophan into kynurenine. Wang et al. [209] has shown when IDO is overexpressed the macrophages differentiation is directed toward the M2 phenotype and once IDO is silenced causing a pro-inflammatory response by macrophages.

The impact of metabolic pathways in macrophage polarization has been investigated in the last years. M1 macrophages rely mostly on glycolysis with a broken TCA cycle that leads to itaconate and succinate accumulation but also present an increase in PPP pathway and fatty acid synthesis (Fig. 3) [158, 167]. On the other hand, M2 macrophages metabolism depends exclusively on OXPHOS and FAO, with an intact TCA cycle [167]. These findings indicate that M1 and M2 macrophages have distinctive metabolic pathways that guarantee their functional phenotype in specific contexts. Finally, the possibility of manipulating macrophages polarization opens a possibility to new therapeutic treatment for clinical applications, although further studies are required for the development of novel therapeutic strategies.

6 Dendritic Cells

Dendritic cells (DC) are professional antigen-presenting cells that are fundamental for the orchestration of immune responses. They are the bridge between innate and adaptive immune responses, inducing the activation and differentiation of naive T lymphocytes [210, 211]. DCs expressed in their surface the pattern recognition receptors (PRRs) which recognize pathogens-associated molecule patterns (PAMPs) and consequently lead to the induction of a general pro-inflammatory response [212]. DCs are divided in three major subsets: conventional DCs (cDC), monocyte-derived DCs (moDC) and plasmacytoid DCs (pDC) are characterized by distinct origins, receptors, and functions [213, 214]. cDCs and pCD derive from BM-derived precursors, whereas inflammatory conditions lead to monocytes recruitment from the blood and

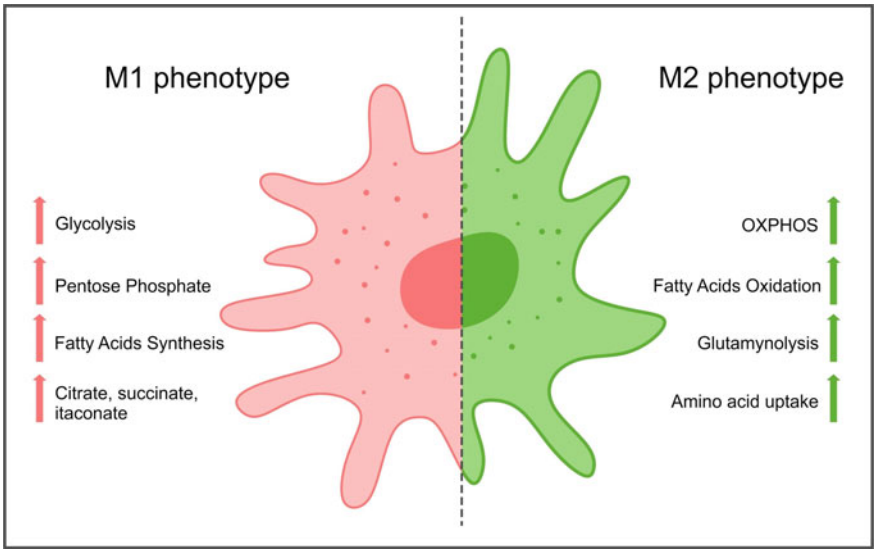


Fig. 3 The metabolic difference in macrophage polarization. During macrophage polarization, M1 macrophages undergo a metabolic reprogramming with increased glycolysis pathways, remarkably with glucose uptake and citrate, succinate and itaconate accumulation and augmented pentose phosphate pathway and fatty acids synthesis to support the proinflammatory phenotype. Whereas, M2 macrophages display enhanced uptake and oxidation of fatty acids, OXPHOS metabolism, amino acid uptake and glutaminolysis sustaining anti-inflammatory phenotype. OXPHOS, oxidative phosphorylation

differentiation into moDC in peripheral tissues like dermis, lung, and intestinal lamina propria [215, 216]. Recent data indicate that cellular metabolism is a fundamental process to regulate DC development and immune response [217, 218].

Like macrophages, DC cells can be activated by recognition of pathogens or danger signals. During the activation process, DC suffers changes in gene expression to support cytokine production and also to present the MHC molecule-loaded peptide epitopes to stimulate T cells [181]. The switch of resting DC to activated DC cells is marked by changes in cellular metabolism, with an increase in bioenergetic demands that are necessary for the maturation process [129, 219]. DC activation upon TLR stimulation causes an increase in glucose consumption, due to the upregulation of glycolysis and the rise in the conversion of pyruvate into lactate [219]. After the activation with TLR, DC promptly increase glycolysis rate [220, 221] and Everts et al. have shown that blocking the glycolysis pathway using 2-DG inhibits the whole activation of DC besides reducing the IL-12 production by DC and, consequently, impairing the ability to stimulate T cells to differentiate in Th1 cells [220–222]. The increase in glycolytic flux during DC activation supplies the citrate demand necessary for this process. After that, citrate is exported to cytosol and converted to acetyl-CoA, an important precursor for fatty acid biosynthesis [218, 223].

Indeed, fatty acid metabolism is an essential pathway for DC development due to the increase in the size of organelles involved in antigen processing and presentation and cytokines synthesis [218, 219]**.** As demonstrated by Ibrahim et al. immunogenicity and the expression of TNFα, IL-6, and TLR are impaired when using an inhibitor of fatty acid synthesis, thus impacting the activation of CD4 T cells and NK cells [224]. Recent data suggest that beyond the synthesis of fatty acids, the storage of total lipids can influence DC function. The TLR stimulation promotes fatty acid synthesis, which increases storage in lipid droplets [221, 225]. Bougnères et al. has demonstrated that blocking the lipid droplet formation inhibits the cross-presentation function DC, impacting CD8 T cell activation [226]. Moreover, analyzes using human and mice DC showed that different concentrations of lipid alter tolerogenic DC responses [224]. DC with high levels of intracellular lipids droplets are capable of inducing more potent pro-inflammatory T cell, NK cell activation whereas DC with low lipid concentration induces regulatory T cell-mediated tolerance [224]. Besides, DC that contains high concentrations of intracellular lipids reduces the proliferation of allogeneic T cells [227, 228].

Also, DC can uptake free fatty acids and oxidize these molecules by the FAO pathway. Stelzner et al. reported that DC can consume free fatty acids, like palmitic acid and oleic acid, and increase the production of IL-23 and IL-1β after LPS stimulation, which could positively impact Th17 differentiation by DC exposed to free fatty acid [229]. Interestingly, the increase in FAO is due to de novo fatty acid synthesis which enhances mitochondrial metabolism [219]**.** It seems that lipid metabolism may have an important role in dictating the tolerogenic status of DC. Wu et al. related that fatty acids pharmacological suppression reduces the TLR-induced production of IFNα, TNFα, and IL-6 by pDCs and also reduces the expression of CD86, a co-stimulatory receptor ligand, impacting in T cell priming [230]. Consistent with these results, Malinarich et al. conducted metabolic analyzes and observed that FAO is an important process for tolerogenic DC development and when this pathway is inhibited prevents the function of tolerogenic DC [231].

7 Concluding Remarks

Metabolism of immune cells under influence of specific external signals, such as antigen recognition, co-stimulatory, and cytokine stimulates different metabolic pathways that can modulate immune cells activation, proliferation, and differentiation [38, 107, 232, 233]. Evidence suggests that immune cell metabolism is essential for the maintenance of homeostasis and even in a disease state, such as cancer and autoimmune diseases [234, 235]. The deprivation or excess of nutrients from the microenvironment modulates both B and T cells, as well as macrophages and DC fate [169, 236, 237]. Issues of particular interest to better understand the potential of and to which extent metabolic pathways and its metabolites impact immune cells will contribute to elucidate immune cell function and even show a great possibility

to become novel therapeutic approaches to suppress several types of inflammatory disease.

The most serious challenge in comprehending the immune profile demand is to understand the metabolic complexity linked to the different immune cell types. Overall, it is increasingly evident that metabolic pathways and their products are essential for the modulation of immune cells functions and fate. Besides, this interplay between metabolism and the immune system represents a new metabolism-targeted for therapeutic strategies related to inflammation and tissue repair, homeostasis and disease progression.

Acknowledgements This study was supported by Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP): grant number: 2019/14755-0. Vinicius Andrade-Oliveira is also a fellow of Pew Latin American Fellow program of the Pew Foundation.

References

1. Gonzalez H, Hagerling C, Werb Z (2018) Roles of the immune system in cancer: from tumor initiation to metastatic progression. Genes Dev 32:1267–1284
2. Nicholson LB (2016) The immune system. Essays Biochem 60:275–301
3. Perdiguero EG, Geissmann F (2016) The development and maintenance of resident macrophages. Nat Immunol 17:28
4. Ginhoux F, Guilliams M (2016) Tissue-resident macrophage ontogeny and homeostasis. Immunity 44:439–449
5. Epelman S, Lavine KJ, Randolph GJ (2014) Origin and functions of tissue macrophages. Immunity 41:21–35
6. Cavaillon J-M (2011) The historical milestones in the understanding of leukocyte biology initiated by Elie Metchnikoff. J Leukoc Biol 90:413–424
7. Medzhitov R (2010) Inflammation 2010: new adventures of an old flame. Cell 140:771–776
8. Watanabe S, Alexander M, Misharin AV, Budinger GRS (2019) The role of macrophages in the resolution of inflammation. J Clin Investig 130:2619–2628
9. Duque GA, Descoteaux a macrophage cytokines: involvement in immunity and infectious diseases
10. Orekhov AN, Orekhova VA, Nikiforov NG, Myasoedova VA, Grechko AV, Romanenko EB, Zhang D, Chistiakov DA (2019) Monocyte differentiation and macrophage polarization. Vessel Plus
11. Wang Y, Smith W, Hao D, He B, Kong L (2019) M1 and M2 macrophage polarization and potentially therapeutic naturally occurring compounds. Int Immunopharmacol 70:459–466
12. Wang T, Liu H, Lian G, Zhang S-Y, Wang X, Jiang C (2017) HIF1-induced glycolysis metabolism is essential to the activation of inflammatory macrophages. Mediat Inflamm 2017:902–9327
13. Ryan DG, ONeill LAJ (2017) Krebs cycle rewired for macrophage and dendritic cell effector functions. FEBS Lett 591:2992–3006
14. Van den Bossche J, Baardman J, Otto NA, van der Velden S, Neele AE, van den Berg SM, Luque-Martin R, Chen H-J, Boshuizen MCS, Ahmed M, Hoeksema MA, de Vos AF, de Winther MPJ (2016) Mitochondrial Dysfunction Prevents Repolarization of Inflammatory Macrophages. Cell Rep 17:684–696
15. Vijayan V, Pradhan P, Braud L, Fuchs HR, Gueler F, Motterlini R, Foresti R, Immenschuh S (2019) Human and murine macrophages exhibit differential metabolic responses to lipopolysaccharide—A divergent role for glycolysis. Redox Biol 22:101–147

16. Tarlinton D (2019) B cells still front and centre in immunology. Nat Rev Immunol 19:85–86
17. Pennock ND, White JT, Cross EW, Cheney EE, Tamburini BA, Kedl RM (2013) T cell responses: naive to memory and everything in between. Adv Physiol Educ 37:273–283
18. Paul WE (2011) Bridging innate and adaptive immunity. Cell 147:1212–1215
19. Olsen Saraiva Camara N, Lepique AP, Basso AS (2012) Lymphocyte differentiation and effector functions. Clin Dev Immunol 2012:510–603
20. Pieper K, Grimbacher B, Eibel H (2013) B-cell biology and development. J Allergy Clin Immunol 131:959–971
21. Melchers F (2015) Checkpoints that control B cell development. J Clin Investig 125:2203–2210
22. Cyster JG, Allen CDC (2019) B cell responses: cell interaction dynamics and decisions. Cell 177:524–540
23. Kumar BV, Connors TJ, Farber DL (2018) Human t cell development, localization, and function throughout life. Immunity 48:202–213
24. Taniuchi I (2018) CD4 helper and CD8 cytotoxic T cell differentiation. Annu Rev Immunol 36:579–601
25. Omilusik KD, Goldrath AW (2017) The origins of memory Tnbsp; cells. Nature 552:337–339
26. Jellusova J (2017) The role of metabolic checkpoint regulators in B cell survival and transformation. [object Object]
27. Waters LR, Ahsan FM, Wolf DM, Shirihai O, Teitell MA (2018) Initial B cell activation induces metabolic reprogramming and mitochondrial remodeling. iScience 5:99–109
28. Doughty CA (2006) Antigen receptor-mediated changes in glucose metabolism in B lymphocytes: role of phosphatidylinositol 3-kinase signaling in the glycolytic control of growth. Blood 107
29. Salmond RJ (2018) mTOR regulation of glycolytic metabolism in T cells. Front cell Dev Biol 6:122
30. Menk AV, Scharping NE, Moreci RS, Zeng X, Guy C, Salvatore S, Bae H, Xie J, Young HA, Wendell SG, Delgoffe GM (2018) Early TCR signaling induces rapid aerobic glycolysis enabling distinct acute T cell effector functions. Cell Rep 22:1509–1521
31. Almeida L, Lochner M, Berod L, Sparwasser T (2016) Metabolic pathways in T cell activation and lineage differentiation. Semin Immunol 28:514–524
32. Wolowczuk I, Verwaerde C, Viltart O, Delanoye A, Delacre M, Pot B, Grangette C (2008) Feeding our immune system: impact on metabolism. Clin Dev Immunol
33. Alwarawrah Y, Kiernan K, Iver NJM (2018) Changes in nutritional status impact immune cell metabolism and function. Front Immunol 9
34. Jung J, Zeng H, Horng T (2019) Metabolism as a guiding force for immunity. Nat Cell Biol 21:85–93
35. Pearce EL, Pearce EJ (2013) Metabolic pathways in immune cell activation and quiescence. Immunity 38:633–643
36. Chapman NM, Boothby MR, Chi H (2019) Metabolic coordination of T cell quiescence and activation. Nat Rev Immunol 20:55–70
37. Guo CA, Bond L, Ntambi JM (2016) Metabolic regulation of inflammation. pp 83105
38. Alves RW, Silva LD, Silva EMD, Furstenau CR, Oliveira VA (2020) The non-canonical role of metabolic enzymes in immune cells and its impact on diseases. Current Tissue Microenviron Rep 1:221–237
39. Lunt SY, Heiden MGV (2011) Aerobic glycolysis: meeting the metabolic requirements of cell proliferation. Annu Rev Cell Dev Biol 27:441–464
40. Donnelly RP, Finlay DK (2015) Glucose, glycolysis and lymphocyte responses. Mol Immunol 68:513–519
41. Reyes IM, Chandel NS (2020) Mitochondrial TCA cycle metabolites control physiology and disease. Nat Commun 11
42. Akram M (2014) Citric acid cycle and role of its intermediates in metabolism. Cell Biochem Biophys 68:47–58

43. Fernie AR, Carrari F, Sweetlove LJ (2004) Respiratory metabolism: glycolysis, the TCA cycle and mitochondrial electron transport. Curr Opin Plant Biol 7:254–261
44. Houten SM, Violante S, Ventura FV, Wanders RJ (2016) The biochemistry and physiology of mitochondrial fatty acid-oxidation and its genetic disorders. Annu Rev Physiol 78:23–44
45. Li Z, Zhang H (2016) Reprogramming of glucose, fatty acid and amino acid metabolism for cancer progression. Cell Mol Life Sci 73:377–392
46. Calder PC (2009) Fatty acids and immune function: relevance to inflammatory bowel diseases. Int Rev Immunol 28:506–534
47. Rose AJ (2019) Amino acid nutrition and metabolism in health and disease. Nutrients 11
48. Gutierrez S, Svahn SL, Johansson ME (2019) Effects of omega-3 fatty acids on immune cells. Int J Mol Sci 20
49. Li P, Yin YL, Li D, Kim SW, Wu G (2007) Amino acids and immune function. Br J Nutr 98:237–252
50. Peng M, Yin N, Chhangawala S, Xu K, Leslie CS, Li MO (2016) Aerobic glycolysis promotes T helper 1 cell differentiation through an epigenetic mechanism. Science 354:481–484
51. Leone RD, Zhao L, Englert JM, Sun IM, Oh MH, Sun IH, Arwood ML, Bettencourt IA, Patel CH, Wen J, Tam A, Blosser RL, Prchalova E, Alt J, Rais R, Slusher BS, Powell JD (2019) Glutamine blockade induces divergent metabolic programs to overcome tumor immune evasion. Science 366:1013–1021
52. Waters LR, Ahsan FM, Wolf DM, Shirihai O, Teitell MA (2018) Initial B cell activation induces metabolic reprogramming and mitochondrial remodeling. IScience 5:99–109
53. Pui JC, Allman D, Xu L, DeRocco S, Karnell FG, Bakkour S, Lee JY, Kadesch T, Hardy RR, Aster JC, Pear WS (1999) Notch1 expression in early lymphopoiesis influences B versus T lineage determination. Immunity 11:299–308
54. Wofford JA, Wieman HL, Jacobs SR, Zhao Y, Rathmell JC (2007) IL-7 promotes Glut1 trafficking and glucose uptake via STAT5-mediated activation of Akt to support T-cell survival. Blood 111:2101–2111
55. Bailis W, Shyer JA, Zhao J, Canaveras JCG, Khazal FJA, Qu R, Steach HR, Bielecki P, Khan O, Jackson R, Kluger Y, Maher LJ, Rabinowitz J, Craft J, Flavell RA (2019) Distinct modes of mitochondrial metabolism uncouple T cell differentiation and function. Nature 571:403–407
56. Chang C-H, Curtis JD, Maggi LB, Faubert B, Villarino AV, OSullivan D, Huang SC-C, van der Windt GJW, Blagih J, Qiu J, Weber JD, Pearce EJ, Jones RG, Pearce EL (2013) Posttranscriptional control of T cell effector function by aerobic glycolysis. Cell 153:1239–1251
57. Ciofani M, Pflucker JCZ (2005) Notch promotes survival of pre-T cells at the beta-selection checkpoint by regulating cellular metabolism. Nat Immunol 6:881–888
58. Tan J, Dudl E, LeRoy E, Murray R, Sprent J, Weinberg K, Surh C (2001) IL-7 is critical for homeostatic proliferation and survival of naive T cells. Proc Natl Acad Sci 98:8732–8737
59. Barrett D, Brown VI, Grupp SA, Teachey DT (2012) Targeting the PI3K/AKT/mTOR signaling axis in children with hematologic malignancies. Pediatr Drugs 14
60. Zeng H, Yang K, Cloer C, Neale G, Vogel P, Chi H (2013) mTORC1 couples immune signals and metabolic programming to establish T(reg)-cell function. Nature 499:48590
61. Michalek RD, Rathmell JC (2010) The metabolic life and times of a T-cell. Immunol Rev 236:190–202
62. Puel A, Ziegler SF, Buckley RH, Leonard WJ (1998) Defective IL7R expression in T(-)B( )NK( ) severe combined immunodeficiency. Nat Genet 20:394–397
63. Magri M, Yatim A, Benne C, Balbo M, Henry A, Serraf A, Sakano S, Gazzolo L, Levy Y, Lelievre JD (2009) Notch ligands potentiate IL-7-driven proliferation and survival of human thymocyte precursors. Eur J Immunol 39:123–140
64. Borlado LR, Barber DF, Hernandez C, Marcos MAR, Sanchez A, Hirsch E, Wymann M, A CM, Carrera AC (2003) Phosphatidylinositol 3-kinase regulates the CD4/CD8 T cell differentiation ratio. J Immunol 170:447–582
65. Jameson SC (2002) Maintaining the norm: T-cell homeostasis. Nat Rev Immunol 2:547–556

66. MacIver NJ, Michalek RD, Rathmell JC (2013) Metabolic regulation of T lymphocytes. Annu Rev Immunol 31:259–283
67. Cole M (1986) The myc oncogene: its role in transformation and differentiation. Annu Rev Genet 20:361–384
68. Warburg O (1925) The Metabolism of Carcinoma Cells. J Cancer Res 9:148–163
69. Wieman HL, Wofford JA, Rathmell JC (2007) Cytokine stimulation promotes glucose uptake via phosphatidylinositol-3 kinase/Akt regulation of Glut1 activity and trafficking. Mol Biol Cell 18:1437–1446
70. Pan Y, Kupper TS (2018) Metabolic reprogramming and longevity of tissue-resident memory T cells. Front Immunol 9
71. Cui G, Staron MM, Gray SM, Ho PC, Amezquita RA, Wu J, Kaech SM (2015) IL-7-induced glycerol transport and TAG synthesis promotes memory CD8 T cell longevity. Cell 161:750–761
72. Yu Q, Erman B, Bhandoola A, Sharrow SO, Singer A (2003) In vitro evidence that cytokine receptor signals are required for differentiation of double positive thymocytes into functionally mature CD8 T cells. J Exp Med 197:475–487
73. Anderson NM, Mucka P, Kern JG, Feng H (2017) The emerging role and targetability of the TCA cycle in cancer metabolism. Protein Cell 9:216–237
74. Gerriets VA, Kishton RJ, Nichols AG, Macintyre AN, Inoue M, Ilkayeva O, Winter PS, Liu X, Priyadharshini B, Slawinska ME, Haeberli L, Huck C, Turka LA, Wood KC, Hale LP, Smith PA, Schneider MA, MacIver NJ, Locasale JW, Newgard CB, Shinohara ML, Rathmell JC (2015) Metabolic programming and PDHK1 control CD4 T cell subsets and inflammation. J Clin Investig 125:194–207
75. Pepper M, Jenkins MK (2011) Origins of CD4( ) effector and central memory T cells. Nat Immunol 12:467–471
76. OSullivan D (2019) The metabolic spectrum of memory T cells. Immunol Cell Biol 97:636–646
77. Pearce EL, Walsh MC, Cejas PJ, Harms GM, Shen H, Wang LS, Jones RG, Choi Y (2009) Enhancing CD8 T-cell memory by modulating fatty acid metabolism. Nature 460:10–37
78. Martin MD, Badovinac VP (2018) Defining Memory CD8 T Cell. Front Immunol 9
79. Nicoli F, Papagno L, Frere JJ, Piccin MPC, Clave E, Gostick E, Toubert A, Price DA, Caputo A, Appay V (2018) Nave CD8 T-cells engage a versatile metabolic program upon activation in humans and differ energetically from memory CD8 T-cells. Front Immunol 9
80. Forster R, Schubel A, Breitfeld D, Kremmer E, Muller IR, Wolf E, Lipp M (1999) CCR7 coordinates the primary immune response by establishing functional microenvironments in secondary lymphoid organs. Cell 99:23–33
81. Loschinski R, Bottcher M, Stoll A, Bruns H, Mackensen A, Mougiakakos D (2018) IL-21 modulates memory and exhaustion phenotype of T-cells in a fatty acid oxidation-dependent manner. Oncotarget 9:1312513138
82. van der Windt GJW, Everts B, Chang C-H, Curtis JD, Freitas TC, Amiel E, Pearce EJ, Pearce EL (2012) Mitochondrial respiratory capacity is a critical regulator of CD8 T cell memory development. Immunity 36:6878
83. Windt GJWVD, Pearce EL (2012) Metabolic switching and fuel choice during T-cell differentiation and memory development. Immunol Rev 249:27–42
84. Wu X, Wu P, Shen Y, Jiang X, Xu F (2018) CD8 Resident memory T cells and viral infection. Front Immunol 9
85. Pan Y, Tian T, Park CO, Lofftus SY, Mei S, Liu X, Luo C, OMalley JT, Gehad A, Teague JE, Divito SJ, Fuhlbrigge R, Puigserver P, Krueger JG, Hotamisligil GS, Clark RA, Kupper TS (2017) Survival of tissue-resident memory T cells requires exogenous lipid uptake and metabolism. Nature 543:252–256
86. Mueller SN, Mackay LK (2015) Tissue-resident memory T cells: local specialists in immune defence. Nat Rev Immunol 16:7989
87. Markman B, Atzori F, Garcia JP, Tabernero J, Baselga J (2009) Status of PI3K inhibition and biomarker development in cancer therapeutics. Ann Oncol 21:683–691

88. Han SJ, Zaretsky AG, Oliveira VA, Collins N, Dzutsev A, Shaik J, Fonseca DMD, Harrison OJ, Tamoutounour S, Byrd AL, Smelkinson M, Bouladoux N, Bliska JB, Brenchley JM, Brodsky IE, Belkaid Y (2017) White adipose tissue is a reservoir for memory T cells and promotes protective memory responses to infection. Immunity 47:1154-1168.e6
89. Zhang Y, Kurupati R, Liu L, Zhou XY, Zhang G, Hudaihed A, Filisio F, Davis WG, Xu X, Karakousis GC, Schuchter LM, Xu W, Amaravadi R, Xiao M, Sadek N, Krepler C, Herlyn M, Freeman GJ, Rabinowitz JD, Ertl HC (2017) Enhancing CD8 T cell fatty acid catabolism withinnbsp; a metabolically challenging tumor microenvironment increases the efficacy of melanoma immunotherapy. Cancer Cell 32:377391.e9
90. Corgnac S, Boutet M, Kfoury M, Naltet C, Chouaib FM (2018) The emerging role of CD8 tissue resident memory T (TRM) cells in antitumor immunity: a unique functional contribution of the CD103 integrin. Front Immunol 9
91. Konjar S, Frising UC, Ferreira C, Hinterleitner R, Mayassi T, Zhang Q, Blankenhaus B, Haberman N, Loo Y, Guedes J, Baptista M, Innocentin S, Stange J, Strathdee D, Jabri B, Veldhoen M (2018) Mitochondria maintain controlled activation state of epithelial-resident T lymphocytes. Sci Immunol 3
92. Teijeira A, Garasa S, Etxeberria I, Gato-Caas M, Melero I, Delgoffe GM (2018) Metabolic consequences of T-cell costimulation in anticancer immunity. Cancer Immunol Res
93. Rathmell J, Heiden MGV, Harris M, Frauwirth K, Thompson C (2000) In the absence of extrinsic signals, nutrient utilization by lymphocytes is insufficient to maintain either cell size or viability. Mol Cell 6:683–692
94. Lochner M, Berod L, Sparwasser T (2015) Fatty acid metabolism in the regulation of T cell function. Trends Immunol 36:81–91
95. Sinclair LV, Rolf J, Emslie E, Shi YB, Taylor PM, Cantrell DA (2013) Control of amino-acid transport by antigen receptors coordinates the metabolic reprogramming essential for T cell differentiation. Nat Immunol 14:500–508
96. Fox CJ, Hammerman PS, Thompson CB (2005) Fuel feeds function: energy metabolism and the T-cell response. Nat Rev Immunol 5:844–852
97. Yusuf I, Fruman DA (2003) Regulation of quiescence in lymphocytes. Trends Immunol 24:380–386
98. Mller AM, Medvinsky A, Strouboulis J, Grosveld F, Dzierzak E (1994) Development of hematopoietic stem cell activity in the mouse embryo. Immunity 1:291–301
99. Melchers F (2005) The pre-B-cell receptor: selector of fitting immunoglobulin heavy chains for the B-cell repertoire. Nat Rev Immunol 5:578–584
100. Victora GD, Nussenzweig MC (2011) Germinal Centers. Annu. Rev. Immunol
101. Kojima H, Kobayashi A, Sakurai D, Kanno Y, Hase H, Takahashi R, Totsuka Y, Semenza GL, Sitkovsky MV, Kobata T (2010) Differentiation stage-specific requirement in hypoxia-inducible factor-1alpha-regulated glycolytic pathway during murine B cell development in bone marrow. J Immunol 184:154–163
102. Jellusova J (2018) Cross-talk between signal transduction and metabolism in B cells. Immunol Lett 201:113
103. Stein M, Dtting S, Mougiakakos D, Bsl M, Fritsch K, Reimer D, Urbanczyk S, Steinmetz T, Schuh W, Bozec A, Winkler TH, Jck H-M, Mielenz D (2017) A defined metabolic state in pre B cells governs B-cell development and is counterbalanced by Swiprosin-2/EFhd1. Cell death Differ 24:1239–1252
104. Maldonado AC, Wang R, Nichols AG, Kuraoka M, Milasta S, Sun LD, Gavin AL, Abel ED, Kelsoe G, Green DR, Rathmell JC (2014) Metabolic reprogramming is required for antibody production that is suppressed in anergic but exaggerated in chronically BAFF-exposed B cells. J Immunol 192:3626–3636
105. Jayachandran N, Mejia EM, Sheikholeslami K, Sher AA, Hou S, Hatch GM, Marshall AJ (2018) TAPP adaptors control B cell metabolism by modulating the phosphatidylinositol 3-kinase signaling pathway: a novel regulatory circuit preventing autoimmunity. J Immunol 201:406–416

106. Diaz-Muoz MD, Bell SE, Fairfax K, Monzon-Casanova E, Cunningham AF, Gonzalez-Porta M, Andrews SR, Bunik VI, Zarnack K, Curk T, Heggermont WA, Heymans S, Gibson GE, Kontoyiannis DL, Ule J, Turner M (2015) The RNA-binding protein HuR is essential for the B cell antibody response. Nat Immunol 16:415–425
107. Jellusova J (2016) Metabolic control of B cell immune responses. [object Object]
108. Dufort FJ, Bleiman BF, Gumina MR, Blair D, Wagner DJ, Roberts MF, Abu-Amer Y, Chiles TC (2007) Cutting edge: IL-4-mediated protection of primary B lymphocytes from apoptosis via Stat6-dependent regulation of glycolytic metabolism. J Immunol 179:4953–4957
109. Li M, Lazorchak AS, Ouyang X, Zhang H, Liu H, Arojo OA, Yan L, Jin J, Han Y, Qu G, Fu Y, Xu X, Liu X, Zhang W, Yang Z, Ruan C, Wang Q, Liu D, Huang C, Lu L, Jiang S, Li F, Su B (2019) Sin1/mTORC2 regulate B cell growth and metabolism by activating mTORC1 and Myc. Cell Mol Immunol
110. Murphy TA, Dang CV, Young JD (2013) Isotopically nonstationary 13C flux analysis of Myc-induced metabolic reprogramming in B-cells. Metab Eng 15:206–217
111. Murphy TA, Dang CV, Young JD (2012) Isotopically nonstationary 13C flux analysis of Myc-induced metabolic reprogramming in B-cells. Metab Eng 15:206–217
112. Mambetsariev N, Lin WW, Wallis AM, Stunz LL, Bishop GA (2016) TRAF3 deficiency promotes metabolic reprogramming in B cells. Sci reports 6:353–349
113. Kurosaki T, Kometani K, Ise W (2015) Memory B cells. Nat Rev Immunol 15:149–159
114. Torigoe M, Iwata S, Nakayamada S, Sakata K, Zhang M, Hajime M, Miyazaki Y, Narisawa M, Ishii K, Shibata H, Tanaka Y (2017) Metabolic reprogramming commits differentiation of human CD27IgD B cells to plasmablasts or CD27IgD cells. J Immunol 199:425–434
115. Deng J, L S, Liu H, Liu B, Jiang C, Xu Q, Feng J, Wang X (2017) Homocysteine activates B cells via regulating PKM2-dependent metabolic reprogramming. J Immunol 198:170–183
116. Brynjolfsson SF, Persson Berg L, Olsen Ekerhult T, Rimkute I, Wick M-J, Mrtensson I-L, Grimsholm O (2018) Long-lived plasma cells in mice and men. Front Immunol 9:2673
117. Blink EJ, Light A, Kallies A, Nutt SL, Hodgkin PD, Tarlinton DM (2002) Early appearance of germinal center-derived memory B cells and plasma cells in blood after primary immunization. J Exp Med 201:545–554
118. Bemark M, Hazanov H, Strmberg A, Komban R, Holmqvist J, Kster S, Mattsson J, Sikora P, Mehr R, Lycke NY (2016) Limited clonal relatedness between gut IgA plasma cells and memory B cells after oral immunization. Nat Commun 7:126–198
119. Kunisawa J (2017) Metabolic changes during B cell differentiation for the production of intestinal IgA antibody. Cell Mol life Sci CMLS 74:1503–1509
120. Kunisawa J, Sugiura Y, Wake T, Nagatake T, Suzuki H, Nagasawa R, Shikata S, Honda K, Hashimoto E, Suzuki Y, Setou M, Suematsu M, Kiyono H (2015) Mode of Bioenergetic Metabolism during B cell differentiation in the intestine determines the distinct requirement for vitamin B1. Cell Rep 13:122–131
121. Caro-Maldonado A, Wang R, Nichols AG, Kuraoka M, Milasta S, Sun LD, Gavin AL, Abel ED, Kelsoe G, Green DR, Rathmell JC (2014) Metabolic reprogramming is required for antibody production that is suppressed in anergic but exaggerated in chronically BAFF-exposed B cells. J Immunol 192:3626–3636
122. Frank RAW, Leeper FJ, Luisi BF (2007) Structure, mechanism and catalytic duality of thiamine-dependent enzymes. Cell Mol life Sci CMLS 64:892–905
123. Lam WY, Becker AM, Kennerly KM, Wong R, Curtis JD, Llufrio EM, McCommis KS, Fahrmann J, Pizzato HA, Nunley RM, Lee J, Wolfgang MJ, Patti GJ, Finck BN, Pearce EL, Bhattacharya D (2016) Mitochondrial pyruvate import promotes long-term survival of antibody-secreting plasma cells. Immunity 45:60–73
124. Nguyen DC, Joyner CJ, Sanz I, Lee FEH (2016) Factors affecting early antibody secreting cell maturation into long-lived plasma cells. [object Object]
125. Yanaba K, Bouaziz JD, Haas KM, Poe JC, Fujimoto M, Tedder TF (2008) A regulatory B cell subset with a unique CD1dhiCD5 phenotype controls T cell-dependent inflammatory responses. Immunity 28:639–650

126. Rosser EC, Mauri C (2015) Regulatory B cells: origin, phenotype, and function. Immunity 42:607–612
127. Mauri C, Bosma A (2012) Immune regulatory function of B cells. Annu Rev Immunol 30:221–241
128. Meng X, Grtsch B, Luo Y, Knaup KX, Wiesener MS, Chen X-X, Jantsch J, Fillatreau S, Schett G, Bozec A (2018) Hypoxia-inducible factor-1 is a critical transcription factor for IL-10-producing B cells in autoimmune disease. Nat Commun 9:251
129. Pearce EL, Pearce EJ (2013) Metabolic pathways in immune cell activation and quiescence. Immunity 38:633–643
130. Ricci J-E, Chiche J (2018) Metabolic reprogramming of non-hodgkins B-cell lymphomas and potential therapeutic strategies. Front Oncol 8:556
131. Caro P, Kishan AU, Norberg E, Stanley IA, Chapuy B, Ficarro SB, Polak K, Tondera D, Gounarides J, Yin H, Zhou F, Green MR, Chen L, Monti S, Marto JA, Shipp MA, Danial NN (2012) Metabolic signatures uncover distinct targets in molecular subsets of diffuse large B cell lymphoma. Cancer cell 22:547–560
132. Afonso J, Pinto T, Simes-Sousa S, Schmitt F, Longatto-Filho A, Pinheiro C, Marques H, Baltazar F (2019) Clinical significance of metabolism-related biomarkers in non-Hodgkin lymphoma—MCT1 as potential target in diffuse large B cell lymphoma. Cell Oncol 42:303–318
133. Monti S, Savage KJ, Kutok JL, Feuerhake F, Kurtin P, Mihm M, Wu B, Pasqualucci L, Neuberg D, Aguiar RCT, Dal Cin P, Ladd C, Pinkus GS, Salles G, Harris NL, Dalla-Favera R, Habermann TM, Aster JC, Golub TR, Shipp MA (2005) Molecular profiling of diffuse large B-cell lymphoma identifies robust subtypes including one characterized by host inflammatory response. Blood 105:1851–1861
134. Ersching J, Efeyan A, Mesin L, Jacobsen JT, Pasqual G, Grabiner BC, Sola DD, Sabatini DM, Victora GD (2017) Germinal center selection and affinity maturation require dynamic regulation of mTORC1 Kinase. Immunity 46:1045-1058.e6
135. Murray PJ, Wynn TA (2011) Protective and pathogenic functions of macrophage subsets. Nat Rev Immunol 11:723–737
136. Sica A, Mantovani A (2012) Macrophage plasticity and polarization: in vivo veritas. J Clin Investig 122:787–795
137. Mosser DM, Edwards JP (2008) Exploring the full spectrum of macrophage activation. Nat Rev Immunol 8:958–969
138. Lawrence T, Natoli G (2011) Transcriptional regulation of macrophage polarization: enabling diversity with identity. Nat Rev Immunol 11:750–761
139. Murray PJ, Wynn TA (2011) Obstacles and opportunities for understanding macrophage polarization. J Leukoc Biol 89:557–563
140. Mills CD, Kincaid K, Alt JM, Heilman MJ, Hill AM (2000) M-1/M-2 macrophages and the Th1/Th2 paradigm. J Immunol 164:6166–6173
141. Rszer T (2015) Understanding the Mysterious M2 Macrophage through Activation Markers and Effector Mechanisms. Mediat Inflamm 2015:8164–8160
142. Palma A, Jarrah AS, Tieri P, Cesareni G, Castiglione F (2018) Gene regulatory network modeling of macrophage differentiation corroborates the continuum hypothesis of polarization states. Front Physiol 9:16–59
143. Wang L-X, Zhang S-X, Wu H-J, Rong X-L, Guo J (2018) M2b macrophage polarization and its roles in diseases. J Leukoc Biol
144. Suzuki H, Hisamatsu T, Chiba S, Mori K, Kitazume MT, Shimamura K, Nakamoto N, Matsuoka K, Ebinuma H, Naganuma M, Kanai T (2016) Glycolytic pathway affects differentiation of human monocytes to regulatory macrophages. Immunol Lett 176:18–27
145. Ecker J, Liebisch G, Englmaier M, Grandl M, Robenek H, Schmitz G (2010) Induction of fatty acid synthesis is a key requirement for phagocytic differentiation of human monocytes. Proc Natl Acad Sci United States Am 107:7817–7822
146. Agha-Jaffar D, Lillycrop KA, Shearmen CP, Calder PC, Burdge GC (2010) Polyunsaturated fatty acid metabolism in monocyte differentiation. Proc Nutr Soc 72

147. Raulien N, Friedrich K, Strobel S, Rubner S, Baumann S, von Bergen M, Krner A, Krueger M, Rossol M, Wagner U (2017) Fatty acid oxidation compensates for lipopolysaccharide-induced Warburg effect in glucose-deprived monocytes. Front Immunol 8:609
148. Diskin C, Plsson-McDermott EM (2018) Metabolic modulation in macrophage effector function. Front Immunol 9:270
149. Torres-Castro I, Arroyo-Camarena D, Martnez-Reyes CP, Gmez-Arauz AY, Dueas-Andrade Y, Hernndez-Ruiz J, Bjar YL, Zaga-Clavellina V, Morales-Montor J, Terrazas LI, Kzhyshkowska J, Escobedo G (2016) Human monocytes and macrophages undergo M1-type inflammatory polarization in response to high levels of glucose. Immunol Lett 176:81–89
150. Vats D, Mukundan L, Odegaard JI, Zhang L, Smith KL, Morel CR, Wagner RA, Greaves DR, Murray PJ, Chawla A (2006) Oxidative metabolism and PGC-1 attenuate macrophage-mediated inflammation. Cell Metab 4
151. Rodrguez-Prados J-C, Travs PG, Cuenca J, Rico D, Aragons J, Martn-Sanz P, Cascante M, Bosc L, Substrate fate in activated macrophages: a comparison between innate, classic, and alternative activation
152. Rodrguez-Prados J-C, Travs PG, Cuenca J, Rico D, Aragons J, Martn-Sanz P, Cascante M, Bosc L (2010) Substrate fate in activated macrophages: a comparison between innate, classic, and alternative activation. J Immunol 185:605–614
153. West AP, Brodsky IE, Rahner C, Woo DK, Erdjument-Bromage H, Tempst P, Walsh MC, Choi Y, Shadel GS, Ghosh S (2011) TLR signalling augments macrophage bactericidal activity through mitochondrial ROS. Nature 472:476–480
154. Huang SC-C, Everts B, Ivanova Y, OSullivan D, Nascimento M, Smith AM, Beatty W, Love-Gregory L, Lam WY, ONeill CM, Yan C, Du H, Abumrad NA, Urban JF, Artyomov MN, Pearce EL, Pearce EJ (2014) Cell-intrinsic lysosomal lipolysis is essential for alternative activation of macrophages. Nat Immunol 15:846–855
155. Zarrinpar A, Bensinger SJ (2017) The therapeutic potential of T cell metabolism. Am J Transpl: Off J Am Soc Transpl Am Soc Transpl Surg 17:17051712
156. ONeill LAJ, Kishton RJ, Rathmell J (2016) A guide to immunometabolism for immunologists. Nat Rev Immunol 16:553–565
157. Hard GC (1970) Some biochemical aspects of the immune macrophage. Br J Exp Pathol 51:97–105
158. Freemerman AJ, Johnson AR, Sacks GN, Milner JJ, Kirk EL, Troester MA, Macintyre AN, Goraksha-Hicks P, Rathmell JC, Makowski L (2014) Metabolic reprogramming of macrophages: glucose transporter 1 (GLUT1)-mediated glucose metabolism drives a proinflammatory phenotype. J Biol Chem 289:7884–7896
159. Pavlou S, Wang L, Xu H, Chen M (2017) Higher phagocytic activity of thioglycollate-elicited peritoneal macrophages is related to metabolic status of the cells. J Inflamm 14:4
160. Tan Z, Xie N, Cui H, Moellering DR, Abraham E, Thannickal VJ, Liu G (2015) Pyruvate dehydrogenase kinase 1 participates in macrophage polarization via regulating glucose metabolism. J Immunol 194:6082–6089
161. Cheon SY, Kim EJ, Kim JM, Kam EH, Ko BW, Koo B-N (2017) Regulation of microglia and macrophage polarization via apoptosis signal-regulating kinase 1 silencing after ischemic/hypoxic injury. Front Mol Neurosci 10:261
162. Huang SC-C, Smith AM, Everts B, Colonna M, Pearce EL, Schilling JD, Pearce EJ (2016) Metabolic reprogramming mediated by the mTORC2-IRF4 signaling axis is essential for macrophage alternative activation. Immunity 45:817–830
163. Wang F, Zhang S, Vuckovic I, Jeon R, Lerman A, Folmes CD, Dzeja PP, Herrmann J (2018) Glycolytic stimulation is not a requirement for M2 macrophage differentiation. Cell Metab 28:463-475.e4
164. Liberti MV, Locasale JW (2016) The warburg effect: how does it benefit cancer cells? Trends Biochem Sci 41:211–218
165. Colegio OR, Chu N-Q, Szabo AL, Chu T, Rhebergen AM, Jairam V, Cyrus N, Brokowski CE, Eisenbarth SC, Phillips GM, Cline GW, Phillips AJ, Medzhitov R (2014) Functional polarization of tumour-associated macrophages by tumour-derived lactic acid. Nature 513:559–563

166. Zhang D, Tang Z, Huang H, Zhou G, Cui C, Weng Y, Liu W, Kim S, Lee S, Perez-Neut M, Czyz D, Hu R, Ye Z, He M, Zheng YG, Shuman H, Ding J, Dai L, Ren B, Roeder RG, Becker L, Zhao Y Metabolic regulation of gene expression by histone lactylation
167. Jha AK, Huang SC-C, Sergushichev A, Lampropoulou V, Ivanova Y, Loginicheva E, Chmielewski K, Stewart KM, Ashall J, Everts B, Pearce EJ, Driggers EM, Artyomov MN (2015) Network integration of parallel metabolic and transcriptional data reveals metabolic modules that regulate macrophage polarization. Immunity 42:419–430
168. Palsson-McDermott EM, Curtis AM, Goel G, Lauterbach MAR, Sheedy FJ, Gleeson LE, van den Bosch MWM, Quinn SR, Domingo-Fernandez R, Johnston DGW, Jiang J-K, Jiang J-K, Israelsen WJ, Keane J, Thomas C, Clish C, Vander Heiden M, Vanden Heiden M, Xavier RJ, ONeill LAJ, (2015) Pyruvate kinase M2 regulates Hif-1 activity and IL-1 induction and is a critical determinant of the warburg effect in LPS-activated macrophages. Cell Metab 21:65–80
169. Viola A, Munari F, Snchez-Rodrguez R, Scolaro T, Castegna A (2019) The metabolic signature of macrophage responses. Front Immunol 10:1462
170. Infantino V, Iacobazzi V, Menga A, Avantaggiati ML, Palmieri F (2014) A key role of the mitochondrial citrate carrier (SLC25A1) in TNF- and IFN-triggered inflammation. Biochim et Biophys Acta 1839:1217–1225
171. Infantino V, Convertini P, Cucci L, Panaro MA, Di Noia MA, Calvello R, Palmieri F, Iacobazzi V (2011) The mitochondrial citrate carrier: a new player in inflammation. Biochem J 438:433–436
172. Infantino V, Iacobazzi V, Palmieri F, Menga A (2013) ATP-citrate lyase is essential for macrophage inflammatory response. Biochem Biophys Res Commun 440:105–111
173. Michelucci A, Cordes T, Ghelfi J, Pailot A, Reiling N, Goldmann O, Binz T, Wegner A, Tallam A, Rausell A, Buttini M, Linster CL, Medina E, Balling R, Hiller K (2013) Immune-responsive gene 1 protein links metabolism to immunity by catalyzing itaconic acid production. Proc Natl Acad Sci United States Am 110:7820–7825
174. Li Y, Zhang P, Wang C, Han C, Meng J, Liu X, Xu S, Li N, Wang Q, Shi X, Cao X (2013) Immune responsive gene 1 (IRG1) promotes endotoxin tolerance by increasing A20 expression in macrophages through reactive oxygen species. J Biol Chem 288:16225–16234
175. Cordes T, Wallace M, Michelucci A, Divakaruni AS, Sapcariu SC, Sousa C, Koseki H, Cabrales P, Murphy AN, Hiller K, Metallo CM (2016) Immunoresponsive Gene 1 and Itaconate inhibit succinate dehydrogenase to modulate intracellular succinate levels. J Biol Chem 291:14274–14284
176. Tannahill GM, Curtis AM, Adamik J, Palsson-McDermott EM, McGettrick AF, Goel G, Frezza C, Bernard NJ, Kelly B, Foley NH, Zheng L, Gardet A, Tong Z, Jany SS, Corr SC, Haneklaus M, Caffrey BE, Pierce K, Walmsley S, Beasley FC, Cummins E, Nizet V, Whyte M, Taylor CT, Lin H, Masters SL, Gottlieb E, Kelly VP, Clish C, Auron PE, Xavier RJ, ONeill LAJ (2013) Succinate is an inflammatory signal that induces IL-1 through HIF-1. Nature 496:238–242
177. Lampropoulou V, Sergushichev A, Bambouskova M, Nair S, Vincent EE, Loginicheva E, Cervantes-Barragan L, Ma X, Huang SC-C, Griss T, Weinheimer CJ, Khader S, Randolph GJ, Pearce EJ, Jones RG, Diwan A, Diamond MS, Artyomov MN (2016) Itaconate links inhibition of succinate dehydrogenase with macrophage metabolic remodeling and regulation of inflammation. Cell Metab 24:158–166
178. Mills EL, Ryan DG, Prag HA, Dikovskaya D, Menon D, Zaslona Z, Jedrychowski MP, Costa ASH, Higgins M, Hams E, Szpyt J, Runtsch MC, King MS, McGouran JF, Fischer R, Kessler BM, McGettrick AF, Hughes MM, Carroll RG, Booty LM, Knatko EV, Meakin PJ, Ashford MLJ, Modis LK, Brunori G, Svin DC, Fallon PG, Caldwell ST, Kunji ERS, Chouchani ET, Frezza C, Dinkova-Kostova AT, Hartley RC, Murphy MP, ONeill LA (2018) Itaconate is an anti-inflammatory metabolite that activates Nrf2 via alkylation of KEAP1. Nature 556:113–117
179. Rubic T, Lametschwandtner G, Jost S, Hinteregger S, Kund J, Carballido-Perrig N, Schwrzler C, Junt T, Voshol H, Meingassner JG, Mao X, Werner G, Rot A, Carballido JM (2008)

Triggering the succinate receptor GPR91 on dendritic cells enhances immunity. Nat Immunol 9:1261–1269
180. He W, Miao FJ-P, Lin DC-H, Schwandner RT, Wang Z, Gao J, Chen J-L, Tian H, Ling L (2004) Citric acid cycle intermediates as ligands for orphan G-protein-coupled receptors. Nature 429:188–193
181. Kelly B, ONeill LAJ (2015) Metabolic reprogramming in macrophages and dendritic cells in innate immunity. Cell Res 25:771–784
182. Littlewood-Evans A, Sarret S, Apfel V, Loesle P, Dawson J, Zhang J, Muller A, Tigani B, Kneuer R, Patel S, Valeaux S, Gommermann N, Rubic-Schneider T, Junt T, Carballido JM (2016) GPR91 senses extracellular succinate released from inflammatory macrophages and exacerbates rheumatoid arthritis. J Exp Med 213:1655–1662
183. van Diepen JA, Robben JH, Hooiveld GJ, Carmone C, Alsady M, Boutens L, Bekkenkamp-Grovenstein M, Hijmans A, Engelke UFH, Wevers RA, Netea MG, Tack CJ, Stienstra R, Deen PMT (2017) SUCNR1-mediated chemotaxis of macrophages aggravates obesity-induced inflammation and diabetes. Diabetologia 60:1304–1313
184. Mills EL, Kelly B, Logan A, Costa ASH, Varma M, Bryant CE, Tourlomousis P, Dbritz JHM, Gottlieb E, Latorre I, Corr SC, McManus G, Ryan D, Jacobs HT, Szibor M, Xavier RJ, Braun T, Frezza C, Murphy MP, ONeill LA (2016) Succinate dehydrogenase supports metabolic repurposing of mitochondria to drive inflammatory macrophages. Cell 167:457–470.e13
185. Cameron AM, Castoldi A, Sanin DE, Flachsmann LJ, Field CS, Puleston DJ, Kyle RL, Patterson AE, Hssler F, Buescher JM, Kelly B, Pearce EL, Pearce EJ (2019) Inflammatory macrophage dependence on NAD salvage is a consequence of reactive oxygen species-mediated DNA damage. Nat Immunol 20:420–432
186. Feng J, Li L, Ou Z, Li Q, Gong B, Zhao Z, Qi W, Zhou T, Zhong J, Cai W, Yang X, Zhao A, Gao G, Yang Z (2018) IL-25 stimulates M2 macrophage polarization and thereby promotes mitochondrial respiratory capacity and lipolysis in adipose tissues against obesity. Cell Mol Immunol 15:493–505
187. Ip WKE, Hoshi N, Shouval DS, Snapper S, Medzhitov R (2017) Anti-inflammatory effect of IL-10 mediated by metabolic reprogramming of macrophages. Science 356:513–519
188. Carneiro FRG, Lepelley A, Seeley JJ, Hayden MS, Ghosh S (2018) An essential role for ECSIT in mitochondrial complex I assembly and mitophagy in macrophages. Cell Rep 22:26542666
189. Ray PD, Huang B-W, Tsuji Y (2012) Reactive oxygen species (ROS) homeostasis and redox regulation in cellular signaling. Cell Signal 24:981–990
190. Baardman J, Verberk SGS, Prange KHM, van Weeghel M, van der Velden S, Ryan DG, Wst RCI, Neele AE, Speijer D, Denis SW, Witte ME, Houtkooper RH, Oneill LA, Knatko EV, Dinkova-Kostova AT, Lutgens E, de Winther MPJ, Van den Bossche J (2018) A defective pentose phosphate pathway reduces inflammatory macrophage responses during hypercholesterolemia. Cell Rep 25:2044-2052.e5
191. Haschemi A, Kosma P, Gille L, Evans CR, Burant CF, Starkl P, Knapp B, Haas R, Schmid JA, Jandl C, Amir S, Lubec G, Park J, Esterbauer H, Bilban M, Brizuela L, Pospisilik JA, Otterbein LE, Wagner O (2012) The sedoheptulose kinase CARKL directs macrophage polarization through control of glucose metabolism. Cell Metab 15:813–826
192. Gonzalez AB, Vidal R, Criollo A, Carreno LJ (2016) New insights on the role of lipid metabolism in the metabolic reprogramming of macrophages. Front Immunol
193. Feingold KR, Shigenaga JK, Kazemi MR, McDonald CM, Patzek SM, Cross AS, Moser A, Grunfeld C (2012) Mechanisms of triglyceride accumulation in activated macrophages. J Leukoc Biol 92:829–839
194. Odegaard JI, Chawla A (2008) Alternative macrophage activation and metabolism. Annu Rev Pathol 6:275–297
195. Malandrino MI, Fucho R, Weber M, Calderon-Dominguez M, Mir JF, Valcarcel L, Escot X, Gmez-Serrano M, Peral B, Salvad L, Fernndez-Veledo S, Casals N, Vzquez-Carrera M, Villarroya F, Vendrell JJ, Serra D, Herrero L (2015) Enhanced fatty acid oxidation in adipocytes and macrophages reduces lipid-induced triglyceride accumulation and inflammation. Am J Physiol Endocrinol Metab 308:E756–E769

196. Huang Y, Morales-Rosado J, Ray J, Myers TG, Kho T, Lu M, Munford RS (2014) Toll-like receptor agonists promote prolonged triglyceride storage in macrophages. J Biol Chem 289:3001–3012
197. Castoldi A, Monteiro LB, Bakker NVT, Sanin DE, Rana N, Corrado M, Cameron AM, Hassler F, Matsushita M, Caputa G, Geltink RIK, Buscher J, Hicks JE, Pearce EL, Pearce EJ (2020) Triacylglycerol synthesis enhances macrophage inflammatory function. Nat Commun 11
198. Newsholme P, Curi R, Gordon S, Newsholme EA (1986) Metabolism of glucose, glutamine, long-chain fatty acids and ketone bodies by murine macrophages. Biochem J 239:121–125
199. Newsholme P, Gordon S, Newsholme EA (1987) Rates of utilization and fates of glucose, glutamine, pyruvate, fatty acids and ketone bodies by mouse macrophages. Biochem J 242:631–636
200. Stunault MI, Bories G, Guinamard RR, Ivanov S (2018) Metabolism plays a key role during macrophage activation. Mediat Inflamm 2018:242–6138
201. Morris SM (2002) Regulation of enzymes of the urea cycle and arginine metabolism. Annu Rev Nutr 22:87–105
202. Hardbower DM, Asim M, Luis PB, Singh K, Barry DP, Yang C, Steeves MA, Cleveland JL, Schneider C, Piazuelo MB, Gobert AP, Wilson KT (2017) Ornithine decarboxylase regulates M1 macrophage activation and mucosal inflammation via histone modifications. Proc Natl Acad Sci United States Am 114:E751–E760
203. Pesce JT, Ramalingam TR, Mentink-Kane MM, Wilson MS, El Kasmi KC, Smith AM, Thompson RW, Cheever AW, Murray PJ, Wynn TA (2009) Arginase-1-expressing macrophages suppress Th2 cytokine-driven inflammation and fibrosis. PLoS Pathog 5:e1000–e1371
204. Murray PJ (2016) Amino acid auxotrophy as a system of immunological control nodes. Nat Immunol 17:132–139
205. Curi R, de Siqueira MR, de Campos Crispin LA, Norata GD, Sampaio SC, Newsholme P (2017) A past and present overview of macrophage metabolism and functional outcomes. Clin Sci 131:1329–1342
206. Meiser J, Krmer L, Sapcariu SC, Battello N, Ghelfi J, DHerouel AF, Skupin A, Hiller K (2016) Pro-inflammatory macrophages sustain pyruvate oxidation through pyruvate dehydrogenase for the synthesis of itaconate and to enable cytokine expression. J Biol Chem 291:3932–3946
207. Liu P-S, Wang H, Li X, Chao T, Teav T, Christen S, Di Conza G, Cheng W-C, Chou C-H, Vavakova M, Muret C, Debackere K, Mazzone M, Huang H-D, Fendt S-M, Ivanisevic J, Ho P-C (2017) Ketoglutarate orchestrates macrophage activation through metabolic and epigenetic reprogramming. Nat Immunol 18:985–994
208. Palmieri EM, Menga A, Martn-Prez R, Quinto A, Riera-Domingo C, De Tullio G, Hooper DC, Lamers WH, Ghesquire B, McVicar DW, Guarini A, Mazzone M, Castegna A (2017) Pharmacologic or genetic targeting of glutamine synthetase skews macrophages toward an M1-like phenotype and inhibits tumor metastasis. Cell Rep 20:1654–1666
209. Wang X-F, Wang H-S, Wang H, Zhang F, Wang K-F, Guo Q, Zhang G, Cai S-H, Du J (2014) The role of indoleamine 2,3-dioxygenase (IDO) in immune tolerance: focus on macrophage polarization of THP-1 cells. Cell Immunol 289:42–48
210. Patente TA, Pelgrom LR, Everts B (2019) Dendritic cells are what they eat: how their metabolism shapes T helper cell polarization. Curr Opin Immunol 58:16–23
211. Giovanelli P, Sandoval TA, Cubillos-Ruiz JR (2019) Dendritic cell metabolism and function in tumors. Trends Immunol
212. Wculek SK, Cueto FJ, Mujal AM, Melero I, Krummel MF, Sancho D (2020) Dendritic cells in cancer immunology and immunotherapy
213. Segura E (2013) Review of mouse and human dendritic cell subsets. Methods Mol Biol 1423:315
214. Patente TA, Pinho MP, Oliveira AA, Evangelista GCM, Bergami-Santos PC, Barbuto JAM (2018) Human dendritic cells: their heterogeneity and clinical application potential in cancer immunotherapy. Front Immunol 9:31–76
215. Collin M, Bigley V (2018) Human dendritic cell subsets: an update. Immunology 154:3–20

216. Schlitzer A, McGovern N, Ginhoux F (2015) Dendritic cells and monocyte-derived cells: two complementary and integrated functional systems. Semin cell Dev Biol 41:9–22
217. Pearce EJ, Everts B (2015) Dendritic cell metabolism. Nat Rev Immunol 15:18–29
218. ONeill LAJ, Pearce EJ (2016) Immunometabolism governs dendritic cell and macrophage function. J Exp Med 213:15–23
219. Du X, Chapman NM, Chi H (2018) Emerging roles of cellular metabolism in regulating dendritic cell subsets and function. Front cell Dev Biol 6:152
220. Everts B, Amiel E, van der Windt GJW, Freitas TC, Chott R, Yarasheski KE, Pearce EL, Pearce EJ (2012) Commitment to glycolysis sustains survival of NO-producing inflammatory dendritic cells. Blood 120:1422–1431
221. Everts B, Amiel E, Huang SC-C, Smith AM, Chang C-H, Lam WY, Redmann V, Freitas TC, Blagih J, van der Windt GJW, Artyomov MN, Jones RG, Pearce EL, Pearce EJ (2014) TLR-driven early glycolytic reprogramming via the kinases TBK1-IKK supports the anabolic demands of dendritic cell activation. Nat Immunol 15:323–332
222. Krawczyk CM, Holowka T, Sun J, Blagih J, Amiel E, DeBerardinis RJ, Cross JR, Jung E, Thompson CB, Jones RG, Pearce EJ (2010) Toll-like receptor-induced changes in glycolytic metabolism regulate dendritic cell activation. Blood 115:4742–4749
223. Ganeshan K, Chawla A (2011) Metabolic regulation of immune responses. Annu Rev Immunol 32:609–634
224. Ibrahim J, Nguyen AH, Rehman A, Ochi A, Jamal M, Graffeo CS, Henning JR, Zambirinis CP, Fallon NC, Barilla R, Badar S, Mitchell A, Rao RS, Acehan D, Frey AB, Miller G (2012) Dendritic cell populations with different concentrations of lipid regulate tolerance and immunity in mouse and human liver. Gastroenterology 143:1061–1072
225. Maroof A, English NR, Bedford PA, Gabrilovich DI, Knight SC (2005) Developing dendritic cells become lacy cells packed with fat and glycogen. Immunology 115:473–483
226. Bougnres L, Helft J, Tiwari S, Vargas P, Chang BH-J, Chan L, Campisi L, Lauvau G, Hugues S, Kumar P, Kamphorst AO, Dumenil A-ML, Nussenzweig M, MacMicking JD, Amigorena S, Guermonprez P (2009) A role for lipid bodies in the cross-presentation of phagocytosed antigens by MHC class I in dendritic cells. Immunity 31:232–244
227. Cao W, Ramakrishnan R, Tuyrin VA, Veglia F, Condamine T, Amoscato A, Mohammadyani D, Johnson JJ, Zhang LM, Klein-Seetharaman J, Celis E, Kagan VE, Gabrilovich DI (2014) Correction: oxidized lipids block antigen cross-presentation by dendritic cells in cancer. J Immunol 192:4935
228. Cubillos-Ruiz JR, Silberman PC, Rutkowski MR, Chopra S, Perales-Puchalt A, Song M, Zhang S, Bettigole SE, Gupta D, Holcomb K, Ellenson LH, Caputo T, Lee A-H, Conejo-Garcia JR, Glimcher LH (2015) ER stress sensor XBP1 controls anti-tumor immunity by disrupting dendritic cell homeostasis. Cell 161:1527–1538
229. Stelzner K, Herbert D, Popkova Y, Lorz A, Schiller J, Gericke M, Klting N, Blher M, Franz S, Simon JC, Saalbach A (2016) Free fatty acids sensitize dendritic cells to amplify TH1/TH17-immune responses. Eur J Immunol 46:2043–2053
230. Wu D, Sanin DE, Everts B, Chen Q, Qiu J, Buck MD, Patterson A, Smith AM, Chang C-H, Liu Z, Artyomov MN, Pearce EL, Cella M, Pearce EJ (2016) Type 1 interferons induce changes in core metabolism that are critical for immune function. Immunity 44:1325–1336
231. Malinarich F, Duan K, Hamid RA, Bijin A, Lin WX, Poidinger M, Fairhurst A-M, Connolly JE (2015) High mitochondrial respiration and glycolytic capacity represent a metabolic phenotype of human tolerogenic dendritic cells. J Immunol 194:5174–5186
232. Thapa B, Lee K (2019) Metabolic influence on macrophage polarization and pathogenesis. BMB Rep 52:360–372
233. Buck MD, OSullivan D, Pearce EL (2015) T cell metabolism drives immunity. J Cell Biol 210
234. Kim J (2018) Regulation of immune cell functions by metabolic reprogramming. J Immunol Res 2018
235. Biswas SK (2015) Metabolic reprogramming of immune cells in cancer progression. Immunity 43:435–449

236. Wculek SK, Khouili SC, Priego E, Murillo IH, Sancho D (2019) Metabolic control of dendritic cell functions: digesting information. Front Immunol 10
237. Franchina DG, Grusdat M, Brenner D (2017) B-cell metabolic remodeling and cancer. Trends Cancer

Metabolic Profile of Innate Immune Cells

Ana Campos Codo, Letícia de Aquino Penteado, Alexandra Ivo de Medeiros, and Pedro Manoel Mendes de Moraes-Vieira

1 Macrophages and Monocytes

Macrophages are immune cells broadly distributed in all tissues of the body and perform key roles in regulating inflammatory responses and tissue homeostasis. There is a large spectrum of macrophages subtypes, which is still evolving [1]. However, to simplify comprehension of how macrophages are metabolically regulated, we are going to divide macrophages in the two opposite extreme phenotypes: M1 or classically activated macrophages and M2 or alternatively activated macrophages. These subtypes are determined in response to environmental stimuli, such as soluble mediators as well as Toll Like Receptor (TLR) ligands. During macrophage polarization several metabolic changes occurs and are critical for macrophages activation and specialization [2, 3].

Codo and Penteado, L.A. contributed equally.
Medeiros and Moraes-Vieira contributed equally.

A. C. Codo · P. M. M. de Moraes-Vieira
Department of Genetics, Microbiology and Immunology, Institute of Biology, Campinas State University, Campinas, Brazil

L. de Aquino Penteado
Department of Biochemistry and Immunology, Ribeirao Preto Medical School, University of São Paulo, Ribeirao Preto, Brazil

L. de Aquino Penteado · A. I. de Medeiros (✉)
Department of Biological Sciences, School of Pharmaceutical Sciences, São Paulo State University, Araraquara, Brazil
e-mail: alexandra.medeiros@unesp.br

P. M. M. de Moraes-Vieira
Experimental Medicine Research Cluster (EMRC), University of Campinas, Sao Paulo, Brazil

N. O. S. Camara et al. (eds.), *Essential Aspects of Immunometabolism in Health and Disease*, https://doi.org/10.1007/978-3-030-86684-6_5

1.1 M1 and M2 Subset Macrophages

1.1.1 M1 Macrophages

M1 macrophages have increased glucose uptake and rely on glycolytic metabolism for ATP production [4]. Mitochondrial respiration and oxidative phosphorylation (OXPHOS) are inhibited in M1 polarized macrophages. The impairment in electron transport chain (ETC) is triggered by nitric oxide (NO), which causes nitrosylation of the iron–sulfur-containing ETC complexes [5] and cannot be reversed [6]. This implicates that macrophages committed to being M1 cannot be reprogrammed to an M2 profile by the addition of interleukin (IL)-4, but M2 macrophages can be converted to M1 profile by stimulus with lipopolysaccharide (LPS) and interferon (IFN)-γ [6]. Also, the activity of complexes I, II and IV are decreased during M1 polarization, which leads to loss of the mitochondrial membrane potential [7].

Upregulated expression of glucose transporter 1 (GLUT1) allows for an increased uptake of glucose [8]. Even though the yield from glycolysis is very low (two molecules of ATP per glucose), this pathway is quickly activated and is essential for ATP production in the absence of OXPHOS to preserve macrophage survival. Upregulation of glycolysis is not only important to maintain high secretory and phagocytic functions of M1 macrophages, but also to fuel the pentose phosphate pathway (PPP) [3]. PPP is essential to the generation of ribose for nucleotides, amino acids for the proteins synthesis, and Nicotinamide Adenine Dinucleotide Phosphate (NADPH) for reactive oxygen species (ROS) production by NADPH oxidase [9].

In glycolysis, the rate-limiting enzyme that converts phosphoenolpyruvate to pyruvate is pyruvate kinase. Pyruvate kinase isoform M2 (PKM2) exists in monomeric, dimeric and tetrameric form [10]. PKM2 is upregulated in M1 macrophages [11] and PKM2 dimer translocate to the nucleus and acts as transcription factor, coactivating hypoxia-inducible factor 1 alpha (HIF-α) and improving the transcription of HIF-1α induced genes. HIF-1α preferentially induces the expression of genes that are related to the glycolytic pathway, besides the transcription of IL-1β and other pro-inflammatory genes [12]. By contrast, tetrameric form of PKM2, which is the catalytic form of PKM2, induced by small-molecules activators, attenuates activation of macrophages induced by LPS and IFN-γ [10].

Pyruvate produced in glycolysis has two outcomes. First, pyruvate can be converted to lactate by the increased expression of lactate dehydrogenase (LDH), diverting metabolism away from the mitochondria. Conversely, pyruvate can also fuel the tricarboxylic acid (TCA) cycle. Activation of the pyruvate dehydrogenase kinase (PDK1) inhibits pyruvate dehydrogenase (PDH) complex by phosphorylation of the E1 subunit, which converts pyruvate in acetyl-CoA that feeds the TCA cycle. PDK1 activation induces an M1 profile in macrophages and knockdown of this enzyme attenuates glycolytic flux [13].

Most of the NAD^+ needed to sustain glycolysis comes from lactate production [2]. However, lactate can lead to macrophage reprogramming, once its intracellular accumulation results in a decrease in glycolytic enzymes and pro-inflammatory

cytokines, such as IL-12, as well as enhances the IL-10 production [14]. Thus, lactate is exported to the extracellular environment by increased expression of Monocarboxylate Transporter 4 (MCT4) [15].

TCA cycle in M1 macrophages is truncated in two places: after citrate and after succinate [16, 17]. Downregulation of isocitrate dehydrogenase, which converts isocitrate to α-ketoglutarate [18] is responsible for the first break in TCA cycle of M1 macrophages [19], which results in high levels of citrate. Citrate is then exported from the mitochondria via the citrate transporter (CIC) and utilized for the fatty acid synthesis (FAS). The increase in FAS is critical to the inflammatory function of M1 macrophages [20], once FAS is required for the plasma membrane remodeling, for example, which is necessary for inflammatory signaling and release of large amounts of cytokines [18]. Also, fatty acids feed the pathways that generate NO and lipid mediators such as leukotrienes, prostaglandins and resolvins [21].

Citrate can also generate itaconic acid, which has direct bactericidal effects and inhibits succinate dehydrogenase (SDH), linking citrate accumulation to succinate accumulation [22]. Because citrate is used for fatty acid synthesis and itaconate production, succinate is originated mainly from glutaminolysis [23]. Succinate accumulation has a direct impact on cytokine production. Under aerobic conditions, prolyl hydroxylases (PHDs) constantly hydroxylate HIF-1α, targeting this protein for proteasomal degradation by VHL E3 complex [24]. VHL deficiency upregulates glycolysis, resulting in epigenetic modifications and enhanced inflammatory and fibrotic responses [25]. However, both succinate and itaconate inhibit PHDs, which leads to stabilization and translocation of HIF-1α into the nucleus even in aerobic conditions. Other consequence of SDH inhibition is the reverse electron transport (RET) [26, 27], which occurs when electrons flow back to complex I, resulting in an increased production of oxidants, which also stabilize HIF-1α [7]. Yet, it has been demonstrated by several groups that succinate oxidation by SDH but not succinate accumulation is critical for inflammatory response [7]. In macrophages depleted of itaconate there is no SDH inhibition, and these cells end up releasing increased levels of IL-1β with higher levels of stabilized HIF-1α than wild-type macrophages [28]. Similarly, macrophages lacking SDH or that received exogenous succinate are less inflammatory than wild-type macrophages [7]. Thus, it appears that HIF-1α is mostly stabilized by ROS instead of succinate.

A key energy/nutrient sensor in macrophage metabolism is the mammalian target of rapamycin (mTOR), a serine/threonine kinase. Assemblage of mTOR with adapter proteins results in two mTOR complexes, mTOR complex 1 (mTORC1) and mTORC2 [29]. Protein synthesis, de novo lipogenesis and glycolysis are importantly shaped by the mTORC1-mTORC2 network [30]. There is an initial peak in protein synthesis after 4 h of LPS stimulation in macrophages that is independent of glucose uptake and glycolysis, relying mostly on phosphatidylinositol 3-kinase (PI3K)-Akt-mTORC1 pathway: after TLR4 activation by LPS, B-cell adapter for PI3K (BCAP) is recruited [31], activating the PI3K pathway, which subsequently triggers Akt and mTORC1 [32]. Activation of Akt also enhances the phosphorylation of hexokinase (HK-II), the enzyme that phosphorylates glucose into glucose-6-phosphate. This phosphorylation results in HK-II association with mitochondria,

where HK-II augments its activity and, therefore, the glycolytic flux [33]. Activation of mTORC1 drives an anabolic response. mTORC1 induces the uptake of glucose through the translocation of HIf-1α and mitochondrial biogenesis. Also, mTORC1 promotes sterol regulatory element-binding proteins (SREBPs)-dependent cholesterol synthesis [34].

Macrophages subsets were first characterized by altered arginine metabolism. This pathway is crucial for macrophage polarization. In M1 macrophages, arginine fuels the NO synthetic pathway, in which inducible nitric oxide synthase (iNOS) mediate the conversion of arginine via citrulline into NO [35]. M1 macrophage polarization requires the iNOS expression and the absence of this enzyme results in defective killing of bacteria and tumor cells, despite reducing the toxicity of septic shock [36].

Several other amino acids can play a crucial role in macrophage activation. Glutamine is required for the induction of IL-1β by LPS-activated macrophages [37]. Stimulation of macrophages with LPS increases the levels of the glutamine transporter Slc3a2 [38]. Mature IL-1β production requires inflammasome activation that can also be metabolic regulated, which can be visualized by the finding that by inducing glycolysis, mTORC1 activates the NLRP3 inflammasome in M1 macrophages [38, 39]. Tryptophan catabolism plays an important role in the immune response against pathogens. In host cells tryptophan prevents the growth of bacteria and parasites [39]. The catabolic metabolism of tryptophan is controlled primarily by an enzyme called indoleamine-2,3-dioxygenase (IDO). IDO catalyzes the initial rate-limiting step of tryptophan degradation and IDO expression is increased in response to LPS stimulus as well as IFN-γ [40, 41]. Despite tryptophan and glutamine, serine metabolism is important for macrophage activation. Serine is necessary for glutathione synthesis, since it is incorporated into glutathione through production of glycine [42]. This synthesis is required for LPS-induced IL-1β expression, which is impaired by serine deprivation; therefore, de novo serine synthesis is essential for the first signal in inflammasome activation [42].

1.1.2 M2 Macrophages

M2 macrophages have an intact TCA cycle and enhanced mitochondrial OXPHOS, which support the phenotype of these cells [43]. Inhibition of mitochondrial ATP synthase suppress IL-4-induced M2 macrophage polarization and function [43].

Mitochondrial oxidative metabolism can be maintained by fatty acid oxidation (FAO), which is preserved by de novo FAS. In M2 macrophages, the scavenger receptor CD36 is responsible to the take up of triacylglycerol-rich lipoproteins, such as LDL and VLDL, which are catabolized in the lysosome by lysosomal acid lipase [44]. Both absence of CD36 and accumulation of triacylglycerol-rich lipoproteins impair M2 activation, showing that FAO together with FAS are important for this macrophage subset [44]. This characterize what is called a futile cycle, in which the substrate is produced and consumed in an intrinsic cycle, but the intermediates have important roles [45]. Nevertheless, it was recently demonstrated that FAO is not essential to M2 polarization. Pyruvate derived from glycolysis can fuel OXPHOS in

M2 macrophages [46]. Yet, it has been demonstrated that inhibition of glycolysis does not compromise M2 markers expression as long as OXPHOS remains functional [47]. This divergence can be explained by the use of 2-deoxy-glucose (2DG) as glycolysis inhibitor. 2DG is a glucose analog that after phosphorylation by HK-2, prevents the continuation of glycolysis. However, 2DG also impairs the carbon flux beyond glycolysis and has an impact on the amount of TCA cycle metabolites and subsequently on OXPHOS [47]. By contrast, galactose replacement suppresses glycolysis without other effects, providing evidence that glycolytic stimulation is not necessary for M2 polarization [47]. Additionaly, glutamine can also fuel the TCA cycle under glucose depletion [26] and can also be used in the hexosamine pathway, resulting in uridine diphosphate N-acetylglucosamine (UDP-GlcNAc) synthesis [48]. UDP-GlcNAc is the substrate for glycosylation of macrophage mannose receptor CD206 and macrophage galactose binding lectin (Mgl1), which are important M2 markers [49].

IL-4 increases the glucose uptake in a time dependent manner and Akt through mTORC1 supports this glucose flux, representing essential regulators of M2 phenotype [50]. However, it has been described that improved glucose metabolism in M2 macrophages is due to IRF4 expression that requires mTORC2 and STAT6 pathway, but not mTORC1 and mTORC1 depletion enhances M2 polarization in macrophages [51]. This disagreement in the literature may be caused by the complexity of mTOR pathway, for example, Akt is downstream of mTORC2 and upstream of mTORC1 [51].

In M2 macrophages, a key nutrient sensor in the immuneregulation is the AMP-activated protein kinase (AMPK). AMPK is a conserved kinase that regulates homeostasis and energetic stress. Activation of AMPK inhibits FAS and cholesterol synthesis [52] while improving FAO and glycolysis [53]. It is described that AMPK is activated in anti-inflammatory macrophages and deactivated by pro-inflammatory stimulus. AMPK can be activated by high AMP/ATP ratio or high intracellular calcium levels. Once activated, AMPK modulates NAD^+/NADH ratio and activates Sirtuin 1, which control IL-12 production in LPS activated macrophages [54].

M2 macrophages have increased expression of arginase-1. Arginase-1 catalyze the conversion of L-arginine into urea and L-ornithine [55]. Ornithine is subsequently used to generate polyamines and proline [55]. Proline is necessary for collagen synthesis, which is instrumental to tissue remodeling and repair mediated by M2 macrophages [56]. Ornithine decarboxylase (ODC), the key enzyme in the polyamine synthesis pathway, converts ornithine in putrescine, which is posteriorly converted in spermidine and spermine [57]. ODC inhibits M1-related gene expression such as iNOS and decrease pro-inflammatory cytokine production, linking polyamine metabolism to M2 macrophage activation [58]. Besides that, several M2 markers, such as CD206, Ym1 and Fizz1 need polyamines for their IL-4-induced expression. This is due the role of polyamines in the regulation of chromatin remodeling through actions in histone methylation and acetylation [59] (Fig. 1).

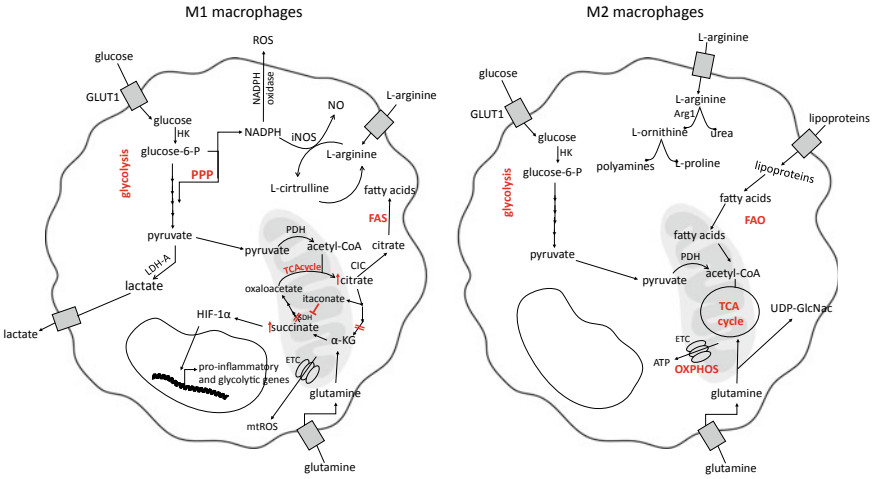


Fig. 1 Metabolism of macrophage subtypes. M1 macrophage metabolism is characterized by enhanced aerobic glycolysis, converting glucose into lactate. M1 macrophages have an increased flux through the pentose phosphate pathway (PPP), producing NADPH, used for the generation of reactive oxygen species (ROS) and nitric oxide (NO). The tricarboxylic acid (TCA) cycle of M1 macrophages is broken in two places, leading to the accumulation of citrate and succinate. Citrate is used for the generation of fatty acids and the synthesis of itaconate, which has microbicidal properties and results in accumulation of succinate. Succinate leads to HIF-1α stabilization and the transcription of pro-inflammatory and glycolytic genes. Another aspect of M1 macrophage metabolism is the conversion of L-arginine to NO. By contrast, M2 macrophages mainly produce ATP through an oxidative TCA cycle coupled to oxidative phosphorylation (OXPHOS). To fuel the TCA cycle, M2 macrophages rely on glycolysis, fatty acid oxidation (FAO) and glutamine metabolism. M2 macrophages show a lowered PPP and convert L-arginine into urea and L-ornithine, which serves as precursor for L-proline and polyamines production

1.2 Immunometabolism in Macrophages Effector Functions

Macrophages are the most important phagocyte involved in the clearance of apoptotic cells, a process called efferocytosis, and is a role that direct contributes to tissue homeostasis. The mechanisms related to apoptotic cell phagocytosis are well described, but metabolic regulation of this process is still poorly understood [60]. Efferocytosis results in elevation of fatty acids consumption by FAO, feeding ETC and activating Sirtuin1 that is required for NAD^+-dependent IL-10 production, thereby polarizing macrophages for tissue repair [61]. Also, the continued clearance of apoptotic cells depends on the reduction of mitochondrial membrane potential [62]. Mitochondrial fission occurs during efferocytosis and is essential for degradation of apoptotic cells in phagolysosomes [63]. Together, these data indicate that macrophage metabolic profile is intrinsically linked to its function.

Another important function of macrophages is their critical role for resolution of infection. Metabolic inhibition of glycolysis markedly decreases the phagocytic

activity of macrophages, which is not affected by the inhibition of oxidative phosphorylation [64]. Thus, the energy required by phagocytosis appears to derive from glycolysis rather than from oxidative phosphorylation. Similar findings are reported about alveolar and peritoneal macrophages [65].

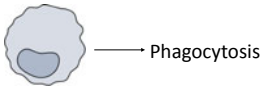
Bone marrow-derived macrophages (BMDMs) or macrophage cell lines are largely used to study metabolism and its link to macrophage function. However, some studies have demonstrated that resident macrophages have a differential behavior. Peritoneal macrophages, for example, are highly glycolytic upon LPS treatment, but also present increased OXPHOS activity, which contrasts the metabolic phenotype of BMDMs [66]. This occurs probably due to delay induction of iNOS in peritoneal macrophages and their enhanced arginase-1 expression, which limits NO production and, consequently, the NO-mediated inhibition of ETC [66]. Besides that, peritoneal macrophages have an increased TCA cycle flux than BMDMs and have a diminished FAS [66].

Alveolar macrophages have enhanced glycerophospholipid metabolism and glycolysis is the main regulator of their responsiveness during IL-4-induced alternative activation [67]. Also, alveolar macrophages have a lower basal metabolism in comparison with BMDMs and require a functional sensing of oxygen for homeostatic functions. This is observed since VHL deficiency, which results in enhanced HIF-1α stabilization, impairs not only terminal differentiation of alveolar macrophages, but also their self-renewal capacity and the handling of surfactants [68].

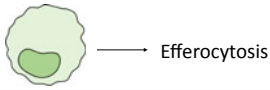
Microglia also has lower metabolic activity at basal state and macrophages from small intestine rely on augmented cholesterol biosynthesis for their effector functions [66]. Therefore, how BMDM correlate with resident macrophage metabolism remains an open field of research (Fig. 2).

2 Monocytes

Monocytes leave the bone marrow, circulate within the peripheral blood and can play important functions including phagocytosis and cytokine production. In the blood, these phagocytes survey the body for sites of inflammation: monocytes rapidly activate and migrate to areas of injury where they can differentiate into macrophages [69]. In human monocytes, the shift from OXPHOS to glycolysis was observed only with LPS activation. In monocytes stimulated with TLR2 agonist, maintenance of phagocytic capacity and cytokine production were dependent on increased OXPHOS [70]. This may be due to the fact that human monocytes produce less amounts of NO when compared with mouse macrophages, therefore, the inhibition of OXPHOS is diminished [66]. The initial enhancement of glycolysis is dependent on HIF-1α, stimulation of Glut1 expression and translocation to the plasma membrane and control of PDK1 activity, which interrupts the pyruvate flux to TCA cycle and favors lactate production [54]. During the progression of acute inflammation into chronic inflammation, monocytes switch their metabolism to rely on FAO [54]. This shift is



Pathways used	
Glycolysis	Supports phagocytosis and pro-inflammatory cytokine production
TCA cycle	Redirected to produce itaconate
PPP	Supports proliferation and ROS production
Amino acid metabolism	Supports proliferation and NO production



Pathways used	
TCA cycle	Supports OXPHOS
OXPHOS	Supports macrophage polarization for tissue repair
FAO	Supports macrophage polarization for tissue repair and inhibits inflammatory signals
Mitochondrial dynamics	Mitochondrial fission is essential for apoptotic cell degradation

Fig. 2 Metabolism over phagocytosis and efferocytosis. Macrophages exhibit a reliance on distinct metabolic pathways to promote cell function. For phagocytosis, macrophages use glycolysis, the tricarboxylic acid (TCA) cycle, the pentose phosphate pathway (PPP) and amino acid metabolism to proliferate and to support the production of inflammatory cytokines. For efferocytosis, macrophages use the TCA cycle, oxidative phosphorylation (OXPHOS), fatty acid oxidation (FAO) and mitochondrial dynamics to inhibit inflammatory signals supporting the polarization for tissue repair

mediated by the proliferator-activated receptor γ coactivators PGC-1α and β, which enhances CD36 and carnitine palmitoyl transferase 1 expression [54].

Different from macrophages, monocytes accumulate cholesterol esters due to lower cholesterol acyltransferase activity, therefore, these cells are more efficient in grasp cholesterol, but less effective in releasing cholesterol [71]. When compared to lymphocytes, monocytes have a lower non-mitochondrial respiration, but higher spare respiratory capacity [72].

Metabolic and epigenetic modification in innate immune system leads to long-term adaptation, which results in hyper or hyporesponsiveness of the cells. This process is called trained immunity and works as a type of innate immune memory [73]. In this context, it has been shown that the production of pro-inflammatory cytokines by monocytes is controlled by glutaminolysis, which modulates gene methylation and results in long-term control [74]. Also, fumarate, which can be generated from aspartate, induces epigenetic modifications in monocytes and together with glycolysis and cholesterol biosynthesis are indispensable for trained immunity [74] (Table 1).

Table 1 Major metabolic pathways in macrophage subsets

	M1 macrophages	M2 macrophages
Glycolysis	Strongly induced, and supports phagocytosis and pro-inflammatory cytokine production	Induced but not crucial for IL-4-induced macrophage activation
TCA cycle	Redirected to produce citrate to promote fatty acid synthesis and succinate to promote HIF-1α-induced gene expression	Support oxidative phosphorylation
OXPHOS	Impaired by NO and itaconate. Electrons flow backwards, driving ROS production	Strongly induced and crucial for IL-4-induced macrophages activation
PPP	Induced and required for ROS generation, NO production, and nucleotide and protein synthesis	Not required
FAS	Supports inflammatory signaling and increases NO and TNF production	Suggested to fuel FAO
FAO	Crucial for inflammasome activation and IL-1β release	Needed to M2 polarization
Amino acid metabolism	Arginine is converted to NO by iNOS. Glutamine fuels the TCA cycle enhancing the succinate accumulation	Arginine is metabolized by arginase-1. Glutamine fuels the TCA cycle and the hexosamine pathway

3 Dendritic Cell Subsets

Dendritic cells (DC) are leukocytes equipped with a wide diversity of pattern recognition receptors (PRRs), specialized in antigen uptake, processing and presentation to naïve T lymphocytes, acting as sentinels in peripheral tissues. These cells have a key role in coordinating both innate and adaptive immune responses. In the presence of microbial products or danger signals, DC rapidly mature, increase the expression of major histocompatibility complex (MHC) molecules as well as co-stimulatory molecules and migrate via afferent lymphatic vessels to the proximal draining lymph node. These mature dendritic cells migrate to lymph node paracortex in order to expose antigenic peptides into MHC molecules that will trigger proper naïve T lymphocytes response [75]. Additionally, under steady-state conditions, tolerogenic DC contribute to homeostasis by sampling self-antigens and promoting regulatory T-cells development [76]. Here, we will discuss the metabolic profile of different DC subsets as well as how intracellular metabolic reprogramming is crucial to determine DC maturation state and function, summarized in (Table 2).

Table 2 Major metabolic pathways in dendritic cell subsets

Metabolic pathway	DC subset	Function
Glycogenolysis	TLR-activated GM-BMDC	To generate glucose-6-phosphate for initial glycolysis
Glycolysis	Mature GM-BMDC	To produce citrate and promote ER/Golgi membrane synthesis
PPP	Mature GM-BMDC	To generate NAPDH and promote ER/Golgi membrane synthesis
Glycolysis	pDC	To support IFN-α production
FAO/OXPHOS	pDC	To generate ATP for DC activation
FAO/OXPHOS	Tol-DC	To induce IDO expression and to inhibit IL-12 and IL-6 production

3.1 Bone-Marrow Dendritic Cells (BMDC) and Monocyte-Derived Dendritic Cells (MoDC)

A widely used protocol to obtain and study DC in vitro consists of culturing murine bone-marrow cells or monocytes derived from peripheral blood with Granulocyte Macrophage Colony-Stimulating Factor (GM-CSF) [77]. The metabolic state of resting DCs is largely dependent on active OXPHOS and FAO [78]. When stimulated with TLR agonists, GM-CSF derived BMDC undergo rapid activation, becoming mature BMDC. After TLR activation with LPS, BMDC rapidly activate glycolysis and associated pathways, such as the PPP, which do not provide a rapid ATP source, but generates citrate and NADPH, thus supporting synthesis of fatty-acids involved in the expansion of the endoplasmic reticulum and Golgi apparatus. Also, BMDC initial switch to glycolysis after TLR stimulation seems to be important for the production and secretion of proteins related to BMDC maturation, such as secretion of IL-12 and the surface expression of MHC class II, CD86 and CCR7 molecules [33]. Although mTOR is a key regulator of anabolic and glycolytic functions [79], the early TLR-induced increase in glycolysis is independent of mTOR and reliant on signaling through TBK1 and IKKε, a downstream cascade involved in TLR activation, leading to PI3K-independent AKT phosphorylation and glycolytic enzyme HK-II association with the mitochondria [33].

As potential carbon sources to fuel TCA cycle and support cell activation, BMDC rely on glucose uptake from extracellular environment, intracellular glycogen reserves or glutamine metabolism [33]. Nevertheless, glutaminolysis inhibition did not have an impact on early BMDC maturation following TLR stimulation, which suggests that this pathway is not involved in the early steps of TLR activation [33]. Moreover, glucose is captured from the extracellular milieu through GLUT1 transporter [80]. However, in BMDC, TLR-mediated glycolytic reprogramming takes place hours before GLUT1 upregulation occurs, which suggests that during TLR activation, BMDC rely on glycogen reserves to fuel TCA cycle and support early glycolytic metabolism driving BMDC maturation [81].

DC must be able to migrate towards the paracortical region of the draining lymph node to activate naïve T cells. The migration ability of BMDC occurs due to upregulation and oligomerization of chemokine receptor CCR7, the receptor for the chemokines CCL21 and CCL19 produced in the paracortical zone of lymph nodes [82]. In this manner, glycolysis seems to be essential for CCR7 surface expression and for BMDC and splenic $CD11c^+$ cDC migratory capability and motility [83].

Regarding the metabolic requirements to sustain DC maturation, LPS stimulation leads to a decrease in mitochondrial oxygen consumption and OXPHOS within 5 h after BMDC activation. This lost in mitochondrial activity coincided with BMDC upregulation of the inducible iNOS expression and NO production, which, as previously mentioned, is a potent inhibitor of the ETC components [84, 85]. In this scenario, BMDCs producing NO must commit to glycolysis to sustain ATP production and cell survival in long-term activation [85]. As previously mentioned, the production of NO is also important for HIF-1α stabilization and, considering that HIF-1α is crucial for the expression of genes encoding glycolytic enzymes, the axis iNOS-NO-HIF-1α represents a positive feedback for glycolysis maintenance [86].

The mitochondrial protein p32 is important to maintain OXPHOS and FAO in resting immature BMDC. Interestingly, deficiency of p32, which results in mitochondrial dysfunction through lack of respiratory chain proteins, leads to reduced BMDC maturation by impaired pyruvate conversion to citrate. Mechanistically, p32 regulates pyruvate dehydrogenase activity. This indicates that mitochondria is actively involved in regulating BMDC maturation [87].

Fc receptors can also induce activation and cytokine production by human MoDC. In particular, Fc gamma receptors (FcγRs), which are receptors for the Fc region of immunoglobulin G (IgG) can synergize with TLR to potentiate DC activation. The FcγR-TLR cross-talk results in transcription factor interferon regulatory factor 5 (IRF5) activation, which boosts the production of pro-inflammatory cytokines by enhancing glycolytic reprogramming in MoDCs, as evidenced by an increase in rates of extracellular acidification (ECAR) and lactate production. This is not only important for DC response and activation to opsonized microorganisms, but also unveil therapeutic targets to attenuate chronic inflammation and for diseases mediated by autoantibody immunocomplexes, such as systemic lupus erythematous (SLE) and rheumatoid arthritis (RA) [88].

Extracellular levels of metabolites and cytokines can also synergize with danger signals received through PRRs to regulate DC function. The Krebs cycle metabolite succinate can act through G protein–coupled receptor GPR91 expressed on immature human-MoDC, which can synergize with TLR activation to potentialize TNF secretion and T cell response mediated by DC activation [89]. Type I IFN are potent anti-viral cytokines and can also play an important role in cDC maturation. Type I IFN signaling through IFNAR stimulation together with TLR agonists induces HIF-1α expression, which guides metabolic transition from OXPHOS to glycolysis [90]. However, contrary to a proinflammatory environment, the presence of the anti-inflammatory cytokine IL-10 leads to AMPK activation, which inhibits BMDC glycolysis and prevents cell maturation [78]. This demonstrates that DC metabolic

reprogramming can also be modulated by environmental cues such as cytokines and metabolites.

Intracellular metabolism pathways are tightly regulated by nutrient/energy sensing molecules that dictates whether anabolic or catabolic metabolism is required. Anabolic metabolism is regulated by mTOR, downstream of PI3K/Akt signaling. In prolonged DC interactions, mTOR seems to play a key role in committing DC to glycolysis and favoring immunogenic DC functions, since rapamycin-mediated mTORC1 inhibition led to impairment of DC activation and enhancement of Tregs cells development [91]. However, long-term activation of mTOR appears to have a negative effect in DC expression of maturation markers, cytokine production and function [92]. mTOR activation can be tightly regulated by the adipokine leptin secreted by adipose tissue and, in turn, mTOR also regulates leptin signaling [93]. Mature BMDC generated from leptin deficient mice expressed lower levels of co-stimulatory molecules and produced less pro-inflammatory cytokines. In this manner, leptin seems to be a crucial contributor during DC maturation, thus linking nutritional status and environmental metabolic cues as considerable influencers of DC metabolism and function [94].

mTOR is also a key regulator of HIF-1α activation which, in turn, is involved in the upregulation of several glycolytic genes and tightly involved with DC activation and proinflammatory functions. Inhibition or loss of HIF-1α impairs co-stimulatory molecule expression and proinflammatory cytokine production by preventing upregulation of glycolytic genes and glycolysis [95]. However, similar to mTOR, long term activation of HIF-1α is also detrimental for BMDC prolonged expression of co-stimulatory molecules and ability to induce CD8 T-cell responses [86].

In contrast to mTOR, AMPK is an important regulator of fatty acid and mitochondria metabolism, upregulating factors involving in OXPHOS and FAO and inhibiting anabolic pathways. Thus, AMPK is suggested to control tolerogenicity of DC, since Dexamethasone/vitamin D3-induced human Tol-moDC upregulated AMPK activity and signaling. Moreover, GM-DC that lack AMPK augmented their proinflammatory phenotype and function when stimulated with LPS [96] (Fig. 3).

3.2 Plasmacytoid Dendritic Cells (pDC)

Plasmacytoid dendritic cells (pDC) are crucial to antivirus immunity, as they preferentially express PRR related to virus recognition, such as TLR7 and TLR9, which senses viral single-stranded RNA and unmethylated CpG-rich DNA, respectively [97]. When activated, mouse splenic pDC are able to produce massive amounts of type I IFN, which depends mostly on PI(3)K-mTOR induction of IRF7 phosphorylation and nuclear localization [98].

The glycolytic pathway seems to be important for pDC function in volunteers vaccinated with live attenuated influenza vaccine, which promotes increased glycolytic rates in primary human pDCs. Viral exposure enhances HIF-1α activation and upregulation of several key glycolytic genes, while OXPHOS pathway genes

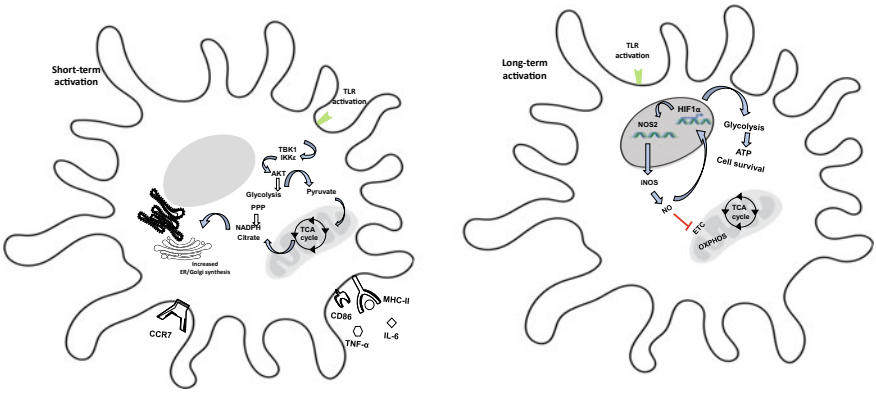


Fig. 3 Dendritic cell depends on glycolysis to mature and survive. Shortly after TLR stimulation, downstream signaling through TBK1 and IKKε leads to AKT phosphorylation and glycolysis/PPP activation. Pyruvate feeds the TCA cycle to generate citrate which, together with NADPH, contributes to increased ER/Golgi synthesis to support DC upregulation of maturation markers and cytokine production. Long-term activated NO-producing DC are strictly dependent on glycolysis for ATP production and survival due to NO inhibition of the electron transport chain (ETC) components and OXPHOS. NO also stabilizes HIF-1α activation, which contributes to iNOS expression and glycolysis maintenance. TLR = Toll-like receptor; PPP = Pentose Phosphate Pathway; TCA = tricarboxylic acid cycle; ER = endoplasmatic reticulum; NO = Nitric oxide

remains unmodulated [99]. In this manner, glycolysis is not only important for pDC upregulation of maturation markers but also for IFN-α secretion, promoting antiviral immunity. As pDCs are considered to be the main producers of type I IFN in SLE, strategies to modulate metabolic pathways in pDC could represent therapeutic targets to improve anti-virus response or even to limit type I IFN–mediated diseases such as SLE [100].

Concomitant, FAO and OXPHOS also seem to have a role in supporting the activation of pDCs sorted from FLT3-L bone marrow cultures. TLR9 agonist leads to upregulation of FAO and OXPHOS pathways, without enhancing glycolysis. The modulation of such pathways was a result from the type I IFN production, which was able to act in an autocrine/paracrine manner to increase FAO and OXPHOS and generate sufficient ATP to support anabolic demands of pDC activation [101] (Fig. 4).

3.3 Tolerogenic DC

While danger signal and microbial products drive DC maturation, tolerogenic signals or the uptake of self-antigens in the absence of a proinflammatory microenvironment impairs DC maturation. These tolerogenic DC (Tol-DC) are characterized by lower expression of co-stimulatory molecules, impaired ability to produce IL-12 while still

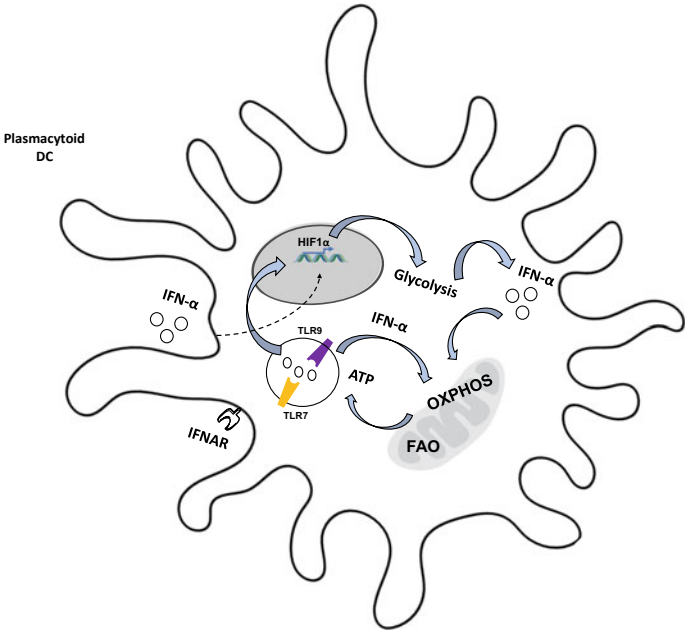


Fig. 4 Plasmacytoid DC depends on glycolysis for type I IFN production. Viral exposure leads to HIF-1α activation and upregulation of several key glycolytic genes, which are important for IFN-α secretion and DC maturation. TLR9 stimulation also upregulates FAO and OXPHOS pathways through type I IFN production, contributing for ATP generation to support DC activation. IFN-α signaling through its receptor also induces HIF-1α expression

able to produce IL-10, TGF-β and express IDO, which contributes to regulatory T cell (Treg) development, thereby promoting peripheral tolerance and maintenance of immune homeostasis [102].

As opposing to the metabolic state of immunogenic DC, the knowledge concerning the metabolism of Tol-DC is rather limited and controversial and comes from the observation of human moDC cultures treated with vitamin D3 (VitD3) and/or dexamethasone (Dex). Dex/vitD3-induced human Tol-moDC have a higher expression of OXPHOS-related genes as well as higher mitochondrial activity and maximal respiratory capacity, compared to LPS-activated MoDC. These Tol-moDC have also increased FAO, which is important to maintain tolerogenic DC function on downregulating activation of allogeneic CD4 T cells [103]. Unexpectedly, these Tol-moDC also show higher glycolytic reserve than LPS-matured moDC, suggesting that tolerogenic moDC maintain their glycolytic machinery and, under OXPHOS restriction, the cells display metabolic plasticity to produce ATP and adapt [103]. Surprisingly, it was also demonstrated that vitamin D3-induced human Tol-moDC

rely on PI3K/Akt/mTOR pathway for the induction and maintenance of their tolerogenic phenotype. In this manner, glycolysis is important for maintaining downregulation of co-stimulatory molecules expression, IL-12 production and inhibition of DC ability to induce CD4 T cell proliferation [104].

The catabolism of essential amino acids also represents a key regulator of tolerance and inflammation. Arginine is metabolized by iNOS under inflammatory conditions, but it can also be alternatively metabolized by arginase 1 (Arg1). Indeed, in response to TGF- β, Arg1 metabolites can induce IDO expression on DC and the catabolism of tryptophan, conferring immunosuppressive effects on DC [105].

The tumor suppressor liver kinase B1 (LKB1) is a key upstream activator of AMPK, which inhibits mTOR and activates catabolic mitochondrial metabolism. LKB1 is able to maintain DCs in a quiescent state by stimulating mitochondrial oxidative metabolism and reducing aerobic glycolysis [106] (Fig. 5).

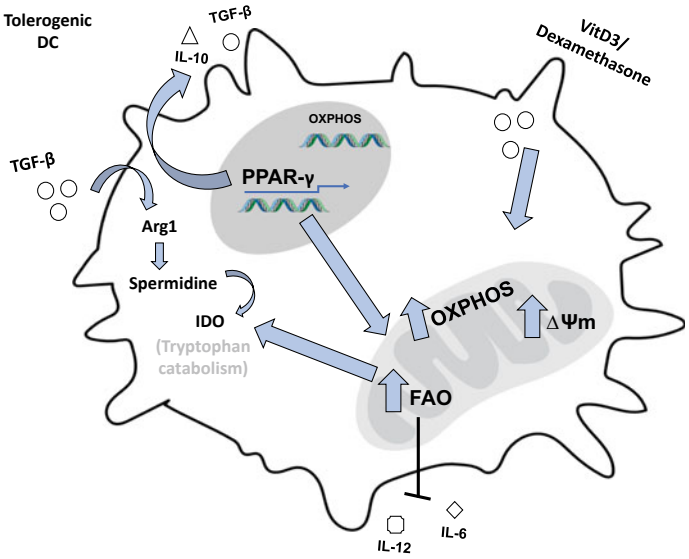


Fig. 5 Tolerogenic DC relies mostly on OXPHOS and FAO metabolism. Tolerogenic signals such as TGF-β and dexamethasone induce PPAR-γ activation and upregulation of genes involved in OXPHOS and FAO, together with an increase in ΔΨm. FAO inhibits IL-12 and IL-6 production. PPAR-γ contributes to IL-10 and TGF-β release. TGF-β upregulates Arg1 expression, which metabolizes arginine to spermidine to induce IDO expression. FAO also contributes to IDO upregulation and tryptophan catabolism. OXPHOS = Oxidative phosphorylation; PPAR-γ = Peroxisome proliferator-activated receptor gamma; VitD3 = Vitamin D3; Arg1 = Arginase 1; IDO = 2,3-dioxygenase; Ψm—mitochondrial membrane potential; FAO = Fatty-acid oxidation

4 Neutrophils

Neutrophils are essential to the process of inflammation and host defense, as they rapidly migrate to the injury or infection sites. During the infection, these inflammatory cells phagocyte and kill the microorganism through different mechanisms that include the release of ROS, proteases and the formation of neutrophil extracellular traps (NET). Unlike macrophage and DC, these phagocytes are short-lived and, within a few hours or days after activation, they undergo cell death. Here, we will address the metabolic profile of activated and aged apoptotic neutrophils as well as the metabolic requirements to perform neutrophil functions.

As neutrophils represent the most abundant leukocyte in blood circulation, these cells have an advantage to arrive first at the injured tissue. Neutrophils must undergo asymmetric changes in cell morphology to migrate in response to chemoattractants in a process termed chemotaxis. Neutrophils are suggested to have fewer mitochondria than any other cell in the immune system, but a growing body of evidence has lightened the role of this organelle in several neutrophil functions. Mitochondria seems to play a pivotal role in mediating neutrophil chemotaxis, as mitochondrial calcium uptake supplies cellular energy demand by enhancing mitochondrial fission, thus providing mitochondrial access in energy-demanding areas, and F-actin accumulation, resulting in cell motility [107]. The chemotactic stimuli triggers mTOR activation, which is responsible for activating mitochondrial calcium uptake and ATP production, favoring neutrophil polarization and chemotaxis. Thus, mitochondria play an important role in host defense by controlling neutrophil chemotaxis to sites of infection and injury [108].

In the site of inflammation, neutrophils phagocytose and kill microorganisms through oxidative and non-oxidative mechanisms, such as degranulation, oxidant production or NET release, which can trap and kill some microorganisms by exposing them to high concentrations of microbicidal factors. NET are composed of DNA, histones and microbicidal peptides and mostly dependent on ROS production to be formed. Neutrophils activation is followed by NET formation and release, resulting in the so-called NETosis, which is a cell death program distinct from apoptosis and necrosis.

NET generation depends on glucose uptake by GLUT1, which expression is enhanced after neutrophil activation. In fact, the energy required for NET formation is mostly derived from glycolysis than mitochondria [109]. However, mitochondrial can also play a key role on NETosis. The NADPH-ROS axis is classically described to regulate NETosis, but there is also a mechanism independent of NADPH which relies on mitochondrial ROS generation, activated by exacerbated increase in intracellular calcium concentrations [110].

Phagocytosis and oxidative burst are also major functions of neutrophils. Mitochondrial ATP production is important for initial cell activation, but neutrophils then switch to glycolysis as the main source of ATP to sustains calcium influx to maintain neutrophil oxidative burst and degranulation [111].

Metabolic profiles of different cellular states of neutrophils showed that, in comparison to LPS-activated cells or unstimulated cells, neutrophils going into spontaneous apoptosis have decreased levels of taurine, which serves as antioxidant and also have a positive effect on neutrophil phagocytosis and killing capacity [112]. However, increased levels of NAD^+ are also observed upon apoptosis with decreased levels of ATP, which might represent an enhanced energy demand or decreased energy production during apoptosis [113]. Also, apoptotic neutrophils were observed to lose their mitochondrial membrane potential before phosphatidylserine surface exposure and changes in cell morphology [114]. In fact, mitochondria do play a role in initiating neutrophil cell death, as these organelles are loaded with proapoptotic proteins, which, upon induction of apoptosis, are released into the cytosol and activate intrinsic death pathway mediated by caspase-9 activation [115] (Fig. 6).

5 Other Granulocytes

Eosinophils are innate immune cells that play an important role against parasites but also promote allergic inflammation [116]. The release of granule proteins, cytokines, chemokines and leukotrienes accomplish a pro-inflammatory role of eosinophils [117]. Eosinophil activation and differentiation are dictated by IL-5, which also function as anti-apoptotic signal [118].

Eosinophils have bioenergetic differences compared to neutrophils, starting by the number of mitochondria present in these cells [115, 119]. Therefore, eosinophils consume more oxygen compared to neutrophils [120]. Eosinophils use glucose oxidation to fuel the TCA cycle. Human eosinophils do not require glutamine and, different from neutrophils, the activation of human eosinophils increases the respiratory capacity of these cells [120]. Therefore, it is suggested that eosinophils might contribute to local hypoxia [121]. During restricted oxygen availability, eosinophils shift to glycolysis which allow them to operate in an inflammatory environment [120]. Therefore, eosinophils are metabolically more flexible than neutrophils and can adapt faster to altered energy conditions. This metabolic profile allows eosinophil to participate in immune homeostasis besides their effect as cytotoxic cell [120] (Fig. 7).

Mast cells are tissue resident myeloid cells that reside in both mucosal and connective tissue and induce inflammatory responses upon surface IgE ligands. These cells are important to perform effector functions in type 2 immunity [122] and are critical for immunity against parasites and venoms; however, they are also involved in the pathology of allergic and atopic diseases [123]. Mast cell response is multiphasic, that is, includes an early release of granules which contain prepackage histamine and TNF-α, and a later phase that includes de novo formation of lipid mediators and production of multiple cytokines, chemokines and other effector molecules [124].

Mast cells use the metabolic pathways to modulate and amplify cellular immune responses and also use metabolic reprogramming to perform their effector functions. Activation of mast cells through FcεR results in a rapidly induction of glycolysis

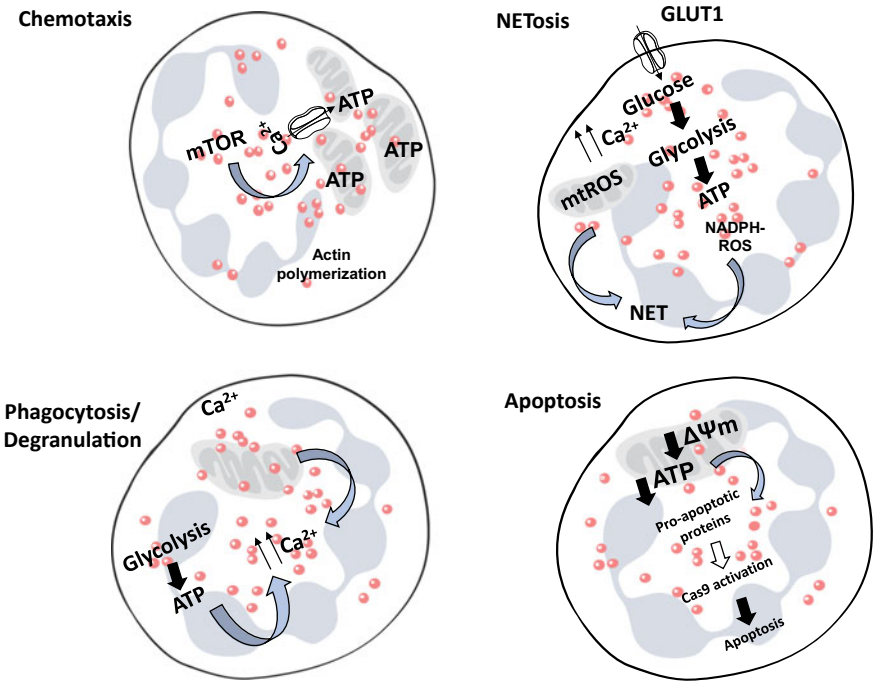


Fig. 6 Metabolism dictates several neutrophil functions. **Chemotaxis**. mTOR induced by chemotactic stimuli leads to mitochondrial calcium uptake, which enhances mitochondrial fission and ATP production to support actin polymerization and neutrophil chemotaxis. **NETosis.** The NADPH-ROS dependent NETosis is also guided by changes in metabolism, as neutrophil enhances glucose uptake by GLUT1 and glycolysis to generate ATP. The increase in intracellular calcium concentration also leads to mitochondrial ROS generation, favouring NETosis in the absence of NADPH. **Phagocytosis and degranulation**. Initial neutrophil activation depends on mitochondrial ATP production. However, glycolysis is the main source of ATP for calcium influx that supports neutrophil function. **Apoptosis**. Neutrophil apoptosis is followed by a decrease in $\Delta\Psi m$ and ATP production. Mitochondrial also releases pro-apoptotic proteins that activate intrinsic death pathway mediated by caspase-9 (cas9) activation

[124]. And lactate inhibits LPS-induced mast cell function by limiting glycolysis and ATP availability [125]. Besides classical activation through FcεR, mast cells function can be regulated by IL-33, a crucial cytokine in allergy and inflammation [126]. Activation with IL-33 increases glycolysis as well provides ATP and ROS for receptor signaling, cytokine production, and effector functions [127]. Direct glycolytic inhibition is sufficient to reduce cytokine production. Although OXPHOS is not required to the initial activation, it is important for mast cells cytokine production, since OXPHOS inhibition drastically decreases this effector function [124]. Similarly, mast cell mitochondria translocate to the site of exocytosis during degranulation and the phosphorylation of mitochondrial STAT3 is responsible for the induction of degranulation, OXPHOS and ATP synthesis [128]. Few mast cells are found in the

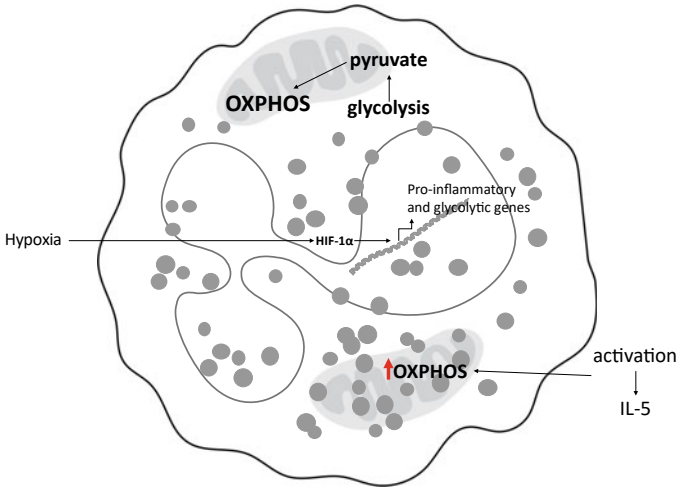


Fig. 7 Eosinophil metabolism. Eosinophils use glucose oxidation to fuel the TCA cycle and OXPHOS. During hypoxia, eosinophils shift to glycolysis which allow them to operate in an inflammatory environment. By contrast, activation of eosinophils increases the respiratory capacity of these cells

adipose tissue of healthy mice and this number increases in adipose tissue inflammation; however, these cells do not contribute to obesity-related metabolic dysregulation [129] (Fig. 8).

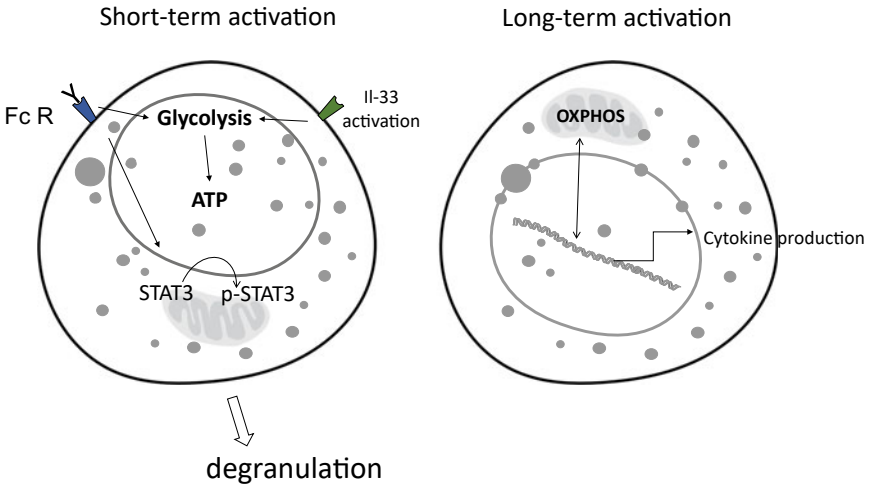


Fig. 8 Mast cell metabolism. Shortly after activation, mast cells upregulate glycolysis, for rapid ATP production. Phosphorylation of mitochondrial STAT3 is responsible for the induction of degranulation, ATP synthesis and OXPHOS. OXPHOS induction is important to support cytokine production in long-term activated mast cells

6 Innate Lymphoid Cells

As tissue-resident cells, innate lymphoid cells (ILC) correspond to T lymphocytes of the innate immune response, only they do not express classical rearranged and diversified antigen receptors like T cells and B cells. Based on their transcriptional factors and cytokine signatures, ILC were divided into 3 groups. Group ILC1s, ILC2s, and ILC3s are the innate counterparts of CD4 T helper (Th)1, Th2, and Th17 cells, respectively. The investigation of ILC metabolic requirements for preserving homeostasis and host defense is rather recent and limited and is summarized in Table 3.

6.1 Group 1—Innate Lymphoid Cells (ILC)-Intrinsic Metabolism

Natural killer (NK) cells, part of an ILC family, are important effector lymphocytes that are characterized for their antiviral and anticancer activities [130]. The innate cytokines that activate NK cells are IL-12, IL-15, and IL-18. They are responsible to direct cytotoxicity of target cells and are potent producers of IFN-γ [131].

Cell-surface markers expression of CD11b and CD27 defines cell maturation in murine NK cells. To facilitate the metabolic demands of proliferation and differentiation, immature NK cells have higher uptake of glucose, higher expression of transferrin receptor and L-amino acid transporter [132]. By contrast, in mature NK cells, FAO and aerobic metabolism metabolic pathways are upregulated, while pathways associated with cell growth are downregulated [133].

Short-term activation of NK cells leads to modest changes in metabolic pathway of these cells, suggesting that, initially, NK cells are prepared to perform their effector functions without considerable metabolic changes [134]. However, the main function of NK cells is at long periods of time past the initial stimulus. Thus, when NK cells are stimulated over extended periods of time, changes in NK cell metabolism are apparent. 3–5 days activation of NK cells with IL-15 upregulates both glycolysis and OXPHOS, which a preferential increase in glycolysis. Inhibition of glycolysis leads to a decrease in NK cell effector function [134]. Despites glycolysis and OXPHOS, the role of other metabolic pathways, as FAO and glutaminolysis, is quite unexplored [134].

Table 3 Major metabolic pathways in innate lymphoid cells subsets

ILC subset	Main metabolic pathways
Immature NK cells	Glycolysis and amino acid metabolism
Mature NK cells	FAO and aerobic metabolism
ILC2	Arginine metabolism and FAO
ILC3	Carbohydrate metabolism and glycolysis

Also, murine NK cells development and maturation has been shown to importantly rely on mTORC1. In mature NK cells, mTORC1 activity is highly upregulated in response to IL-15 or IL-2 and it is required to improved glycolysis [133]. Besides mTORC1, the transcription factor cMyc is crucial for murine NK cell metabolic and functional responses. cMyc protein levels are upregulated by amino acids: cMyc expression is rapidly lost when L-amino acid transport is blocked or when glutamine is withdrawn [135].

In human NK cells, the expression of the cell-surface marker CD56 defines cell maturation. mTORC1 is also a key regulator of metabolic responses and IL-2 can induce mTORC1 activity [136]. However, the exact role for mTORC1 in human NK cells requires more studies because it has been shown that mTORC1 is not required for IL-12/IL-15-induced metabolic responses [137].

6.2 Group 2—ILC-Intrinsic Metabolism

Representing a primary source of tissue IL-5, IL-9 and IL-13, ILC2 are important for mucosal barrier immunity against allergic and nonallergic threats [138]. Upon type 2 inflammation, activated ILC2 augments mitochondrial respiration together with aerobic glycolysis and accumulate in the tissue in response to IL-33 [139]. Amino acid metabolism seems to be crucial in regulating ILC2. In the lung, under resting conditions, ILC2 constitutively express Arg1 in a STAT6-independent manner [140]. Arg1 metabolizes the amino acid L-arginine to generate metabolites to sustain cell proliferation, amongst other functions. The role of Arg1 in ILC2 proliferative capacity, tough, remains contradictory. Deletion of arginase-1 did not impact lung ILC2 proliferation or cytokine production during helminth infection [140]. However, an alternative strategy to delete Arg1 in ILC2 demonstrated that Arg1 deficiency reduces ILC2 optimal proliferation and cytokine production, impairing type 2 inflammation in lungs [139]. This suggests that, upon activation, ILC2 requires Arg1 expression to activate L-arginine catabolism and conversion into polyamines that support glycolysis required for cell growth, proliferation and cytokine production [139]. The differences in strategies to delete Arg1 in ILC2 might explain the opposing effects regarding Arg1 role in ILC2 proliferation and function.

VHL deficiency (which results in the accumulation of HIF-1α) impaired ILC2 development and accumulation/activation in the lung as well as attenuated the expression of the IL-33 receptor [141]. This deficiency also resulted in increased glycolysis with reduced mitochondrial respiration. Thus, VHL play a key part in regulating ILC2 activation and function through regulation of HIF-1α dependent commitment to glycolysis [141].

A gene expression analysis of ILCs revealed that ILC2 expressed several genes related to lipid metabolism. In addition to maintaining mucosal immunity, ILC2 reside in visceral adipose tissue, where they can also regulate adipose tissue homeostasis [142]. Under homeostatic conditions, intestinal ILC2 capture fatty acids (FA) over glucose from the environment. Following a helminth infection, FAO blockage or inhibition of FA intestinal absorption prevented ILC2 accumulation and cytokine production, suggesting that uptake of FA and FAO are important for ILC2 activation in the context of intestinal helminth infection [143].

6.3 Group 3 ILC-Intrinsic Metabolism

Group 3 ILC are important in host defense against extracellular bacteria and fungi, was well as in the tolerance to microbiota. They can produce large amounts of IL-22, thus maintaining intestinal homeostasis. Little is known about ILC3 metabolism. Like ILC2, ILC3 cells can also capture extracellular FA [143] and, although Arg1 is also expressed on ILC3, they do not depend on Arg1 to develop and perform cellular function in the steady state or in the context of intestinal inflammation [139].

Transcriptional profiling of ILC residing in the small intestinal lamina propria revealed that ILC3 expressed a gene signature related to carbohydrate metabolism, such as fructose, mannose, and galactose metabolism in addition to glycolysis, [144], which is also a feature of effector T lymphocytes [145].

Additionally, the mTOR-HIF-1α axis is important for preserving cytokine production and cell proliferation, as pharmacological inhibition or mTORC1 deletion compromises ILC3-mediated host defense against *C. rodentium infecton.* Mechanistically, mTOR induces HIF-1α which, in turn, favors glycolysis, ILC3 activation and Rorγt expression [146].

Interestingly, despite HIF-1α role in decreasing mitochondrial oxidative metabolism in Th17 cells, this is not the case for ILC3. mTORC1 seems to activate mitochondrial metabolism, evidenced by increased cell oxygen consumption and mitochondrial ROS production which, in turn, supports HIF-1α activity and ILC3 functions. Moreover, ILC3 do not solely depends on glycolysis, as etomoxir treatment blunted IL-17A and IL-22 production, thus ILC3 are also capable to use lipids as fuel [146] (Fig. 9).

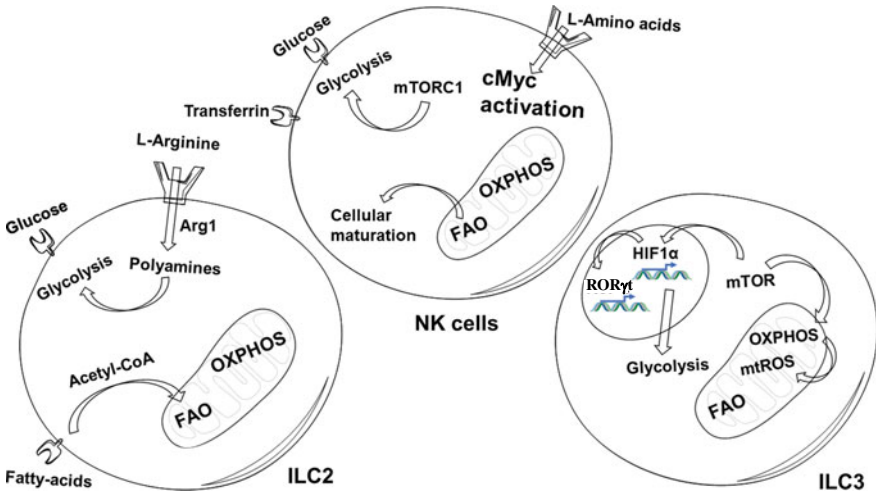


Fig. 9 Metabolic programs active in innate lymphoid cells. NK cells rely mostly on glycolysis and OXPHOS for activation and cytokine production, being FAO important for cellular maturation. mTORC1 is important for maintenance of glycolysis in these cells. NK cells also have enhanced expression of transferrin and L-amino acids receptors. cMyc is crucial for functional responses of NK cells. ILC2 cells depend largely on Arg1 conversion of L-arginine into polyamines to support glycolysis, cell proliferation and cytokine production. Moreover, ILC2 residing in visceral adipose tissues are able to capture fatty acids from the environment, thus upregulating lipid metabolism and FAO to sustain ILC2 regulation of adipose tissue homeostasis. ILC3 cells depend on carbohydrate metabolism and mTOR induction of HIF-1α to upregulate glycolysis and support Rorγt expression. mTOR also upregulates OXPHOS and mtROS production to regulate HIF-1α expression and ILC3 cytokine production

References

1. Xue J, Schmidt SV, Sander J, Draffehn A, Krebs W, Quester I, De Nardo D, Gohel TD, Emde M, Schmidleithner L, Ganesan H, Nino-Castro A, Mallmann MR, Labzin L, Theis H, Kraut M, Beyer M, Latz E, Freeman TC, Ulas T, Schultze JL (2014) Transcriptome-based network analysis reveals a spectrum model of human macrophage activation. Immunity 40(2):274–288. https://doi.org/10.1016/j.immuni.2014.01.006
2. O'Neill LA, Kishton RJ, Rathmell J (2016) A guide to immunometabolism for immunologists. Nat Rev Immunol 16(9):553–565. https://doi.org/10.1038/nri.2016.70
3. Van den Bossche J, O'Neill LA, Menon D (2017) Macrophage Immunometabolism: where are we (going)? Trends Immunol 38(6):395–406. https://doi.org/10.1016/j.it.2017.03.001
4. Kelly B, O'Neill LA (2015) Metabolic reprogramming in macrophages and dendritic cells in innate immunity. Cell Res 25(7):771–784. https://doi.org/10.1038/cr.2015.68
5. Clementi E, Brown GC, Feelisch M, Moncada S (1998) Persistent inhibition of cell respiration by nitric oxide: crucial role of S-nitrosylation of mitochondrial complex I and protective action of glutathione. Proc Natl Acad Sci USA 95(13):7631–7636. https://doi.org/10.1073/pnas.95.13.7631
6. Van den Bossche J, Baardman J, Otto NA, van der Velden S, Neele AE, van den Berg SM, Luque-Martin R, Chen HJ, Boshuizen MC, Ahmed M, Hoeksema MA, de Vos AF, de Winther MP (2016) Mitochondrial dysfunction prevents repolarization of inflammatory macrophages. Cell Rep 17(3):684–696. https://doi.org/10.1016/j.celrep.2016.09.008

7. Mills EL, Kelly B, Logan A, Costa ASH, Varma M, Bryant CE, Tourlomousis P, Dabritz JHM, Gottlieb E, Latorre I, Corr SC, McManus G, Ryan D, Jacobs HT, Szibor M, Xavier RJ, Braun T, Frezza C, Murphy MP, O'Neill LA (2016) Succinate dehydrogenase supports metabolic repurposing of mitochondria to drive inflammatory macrophages. Cell 167 (2):457–470 e413. https://doi.org/10.1016/j.cell.2016.08.064
8. Freemerman AJ, Johnson AR, Sacks GN, Milner JJ, Kirk EL, Troester MA, Macintyre AN, Goraksha-Hicks P, Rathmell JC, Makowski L (2014) Metabolic reprogramming of macrophages: glucose transporter 1 (GLUT1)-mediated glucose metabolism drives a proinflammatory phenotype. J Biol Chem 289(11):7884–7896. https://doi.org/10.1074/jbc.M113.522037
9. Nagy C, Haschemi A (2015) Time and demand are two critical dimensions of immunometabolism: the process of macrophage activation and the pentose phosphate pathway. Front Immunol 6:164. https://doi.org/10.3389/fimmu.2015.00164
10. Palsson-McDermott EM, Curtis AM, Goel G, Lauterbach MA, Sheedy FJ, Gleeson LE, van den Bosch MW, Quinn SR, Domingo-Fernandez R, Johnston DG, Jiang JK, Israelsen WJ, Keane J, Thomas C, Clish C, Vander Heiden M, Xavier RJ, O'Neill LA (2015) Pyruvate kinase M2 regulates Hif-1alpha activity and IL-1beta induction and is a critical determinant of the warburg effect in LPS-activated macrophages. Cell Metab 21(1):65–80. https://doi.org/10.1016/j.cmet.2014.12.005
11. El Kasmi KC, Stenmark KR (2015) Contribution of metabolic reprogramming to macrophage plasticity and function. Semin Immunol 27(4):267–275. https://doi.org/10.1016/j.smim.2015.09.001
12. Tannahill GM, Curtis AM, Adamik J, Palsson-McDermott EM, McGettrick AF, Goel G, Frezza C, Bernard NJ, Kelly B, Foley NH, Zheng L, Gardet A, Tong Z, Jany SS, Corr SC, Haneklaus M, Caffrey BE, Pierce K, Walmsley S, Beasley FC, Cummins E, Nizet V, Whyte M, Taylor CT, Lin H, Masters SL, Gottlieb E, Kelly VP, Clish C, Auron PE, Xavier RJ, O'Neill LA (2013) Succinate is an inflammatory signal that induces IL-1beta through HIF-1alpha. Nature 496(7444):238–242. https://doi.org/10.1038/nature11986
13. Tan Z, Xie N, Cui H, Moellering DR, Abraham E, Thannickal VJ, Liu G (2015) Pyruvate dehydrogenase kinase 1 participates in macrophage polarization via regulating glucose metabolism. J Immunol 194(12):6082–6089. https://doi.org/10.4049/jimmunol.1402469
14. Tan Z, Xie N, Banerjee S, Cui H, Fu M, Thannickal VJ, Liu G (2015) The monocarboxylate transporter 4 is required for glycolytic reprogramming and inflammatory response in macrophages. J Biol Chem 290(1):46–55. https://doi.org/10.1074/jbc.M114.603589
15. Petersen C, Nielsen MD, Andersen ES, Basse AL, Isidor MS, Markussen LK, Viuff BM, Lambert IH, Hansen JB, Pedersen SF (2017) MCT1 and MCT4 expression and lactate flux activity increase during white and brown adipogenesis and impact adipocyte metabolism. Sci Rep 7(1):13101. https://doi.org/10.1038/s41598-017-13298-z
16. Jha AK, Huang SC, Sergushichev A, Lampropoulou V, Ivanova Y, Loginicheva E, Chmielewski K, Stewart KM, Ashall J, Everts B, Pearce EJ, Driggers EM, Artyomov MN (2015) Network integration of parallel metabolic and transcriptional data reveals metabolic modules that regulate macrophage polarization. Immunity 42(3):419–430. https://doi.org/10.1016/j.immuni.2015.02.005
17. O'Neill LA (2015) A broken krebs cycle in macrophages. Immunity 42(3):393–394. https://doi.org/10.1016/j.immuni.2015.02.017
18. Wei X, Song H, Yin L, Rizzo MG, Sidhu R, Covey DF, Ory DS, Semenkovich CF (2016) Fatty acid synthesis configures the plasma membrane for inflammation in diabetes. Nature 539(7628):294–298. https://doi.org/10.1038/nature20117
19. Infantino V, Iacobazzi V, Palmieri F, Menga A (2013) ATP-citrate lyase is essential for macrophage inflammatory response. Biochem Biophys Res Commun 440(1):105–111. https://doi.org/10.1016/j.bbrc.2013.09.037
20. Infantino V, Convertini P, Cucci L, Panaro MA, Di Noia MA, Calvello R, Palmieri F, Iacobazzi V (2011) The mitochondrial citrate carrier: a new player in inflammation. Biochem J 438(3):433–436. https://doi.org/10.1042/BJ20111275

21. Williams NC, O'Neill LAJ (2018) A role for the Krebs cycle intermediate citrate in metabolic reprogramming in innate immunity and inflammation. Front Immunol 9:141. https://doi.org/10.3389/fimmu.2018.00141
22. McFadden BA, Purohit S (1977) Itaconate, an isocitrate lyase-directed inhibitor in Pseudomonas indigofera. J Bacteriol 131(1):136–144
23. Mills EL, Ryan DG, Prag HA, Dikovskaya D, Menon D, Zaslona Z, Jedrychowski MP, Costa ASH, Higgins M, Hams E, Szpyt J, Runtsch MC, King MS, McGouran JF, Fischer R, Kessler BM, McGettrick AF, Hughes MM, Carroll RG, Booty LM, Knatko EV, Meakin PJ, Ashford MLJ, Modis LK, Brunori G, Sevin DC, Fallon PG, Caldwell ST, Kunji ERS, Chouchani ET, Frezza C, Dinkova-Kostova AT, Hartley RC, Murphy MP, O'Neill LA (2018) Itaconate is an anti-inflammatory metabolite that activates Nrf2 via alkylation of KEAP1. Nature 556(7699):113–117. https://doi.org/10.1038/nature25986
24. Haase VH (2006) The VHL/HIF oxygen-sensing pathway and its relevance to kidney disease. Kidney Int 69(8):1302–1307. https://doi.org/10.1038/sj.ki.5000221
25. Zhang W, Li Q, Li D, Li J, Aki D, Liu YC (2018) The E3 ligase VHL controls alveolar macrophage function via metabolic-epigenetic regulation. J Exp Med 215(12):3180–3193. https://doi.org/10.1084/jem.20181211
26. Liu PS, Wang H, Li X, Chao T, Teav T, Christen S, Di Conza G, Cheng WC, Chou CH, Vavakova M, Muret C, Debackere K, Mazzone M, Huang HD, Fendt SM, Ivanisevic J, Ho PC (2017) alpha-ketoglutarate orchestrates macrophage activation through metabolic and epigenetic reprogramming. Nat Immunol 18(9):985–994. https://doi.org/10.1038/ni.3796
27. Murphy MP, O'Neill LAJ (2018) Krebs cycle reimagined: the emerging roles of succinate and itaconate as signal transducers. Cell 174(4):780–784. https://doi.org/10.1016/j.cell.2018.07.030
28. Lampropoulou V, Sergushichev A, Bambouskova M, Nair S, Vincent EE, Loginicheva E, Cervantes-Barragan L, Ma X, Huang SC, Griss T, Weinheimer CJ, Khader S, Randolph GJ, Pearce EJ, Jones RG, Diwan A, Diamond MS, Artyomov MN (2016) Itaconate links inhibition of succinate dehydrogenase with macrophage metabolic remodeling and regulation of inflammation. Cell Metab 24(1):158–166. https://doi.org/10.1016/j.cmet.2016.06.004
29. Weichhart T, Hengstschlager M, Linke M (2015) Regulation of innate immune cell function by mTOR. Nat Rev Immunol 15(10):599–614. https://doi.org/10.1038/nri3901
30. Byles V, Covarrubias AJ, Ben-Sahra I, Lamming DW, Sabatini DM, Manning BD, Horng T (2013) The TSC-mTOR pathway regulates macrophage polarization. Nat Commun 4:2834. https://doi.org/10.1038/ncomms3834
31. Troutman TD, Hu W, Fulenchek S, Yamazaki T, Kurosaki T, Bazan JF, Pasare C (2012) Role for B-cell adapter for PI3K (BCAP) as a signaling adapter linking Toll-like receptors (TLRs) to serine/threonine kinases PI3K/Akt. Proc Natl Acad Sci USA 109(1):273–278. https://doi.org/10.1073/pnas.1118579109
32. Ivanov SS, Roy CR (2013) Pathogen signatures activate a ubiquitination pathway that modulates the function of the metabolic checkpoint kinase mTOR. Nat Immunol 14(12):1219–1228. https://doi.org/10.1038/ni.2740
33. Everts B, Amiel E, Huang SC, Smith AM, Chang CH, Lam WY, Redmann V, Freitas TC, Blagih J, van der Windt GJ, Artyomov MN, Jones RG, Pearce EL, Pearce EJ (2014) TLR-driven early glycolytic reprogramming via the kinases TBK1-IKKvarepsilon supports the anabolic demands of dendritic cell activation. Nat Immunol 15(4):323–332. https://doi.org/10.1038/ni.2833
34. Inoki K, Kim J, Guan KL (2012) AMPK and mTOR in cellular energy homeostasis and drug targets. Annu Rev Pharmacol Toxicol 52:381–400. https://doi.org/10.1146/annurev-pharmtox-010611-134537
35. MacMicking J, Xie QW, Nathan C (1997) Nitric oxide and macrophage function. Annu Rev Immunol 15:323–350. https://doi.org/10.1146/annurev.immunol.15.1.323
36. MacMicking JD, Nathan C, Hom G, Chartrain N, Fletcher DS, Trumbauer M, Stevens K, Xie QW, Sokol K, Hutchinson N et al (1995) Altered responses to bacterial infection and endotoxic shock in mice lacking inducible nitric oxide synthase. Cell 81(4):641–650

37. Ren W, Xia Y, Chen S, Wu G, Bazer FW, Zhou B, Tan B, Zhu G, Deng J, Yin Y (2019) Glutamine metabolism in macrophages: a novel target for obesity/type 2 diabetes. Adv Nutr 10(2):321–330. https://doi.org/10.1093/advances/nmy084
38. Tavakoli S, Downs K, Short JD, Nguyen HN, Lai Y, Jerabek PA, Goins B, Toczek J, Sadeghi MM, Asmis R (2017) Characterization of macrophage polarization states using combined measurement of 2-deoxyglucose and glutamine accumulation: implications for imaging of atherosclerosis. Arterioscler Thromb Vasc Biol 37(10):1840–1848. https://doi.org/10.1161/ATVBAHA.117.308848
39. Moon JS, Hisata S, Park MA, DeNicola GM, Ryter SW, Nakahira K, Choi AMK (2015) mTORC1-induced HK1-dependent glycolysis regulates NLRP3 inflammasome activation. Cell Rep 12(1):102–115. https://doi.org/10.1016/j.celrep.2015.05.046
40. Yoshida R, Hayaishi O (1978) Induction of pulmonary indoleamine 2,3-dioxygenase by intraperitoneal injection of bacterial lipopolysaccharide. Proc Natl Acad Sci USA 75(8):3998–4000. https://doi.org/10.1073/pnas.75.8.3998
41. Yoshida R, Imanishi J, Oku T, Kishida T, Hayaishi O (1981) Induction of pulmonary indoleamine 2,3-dioxygenase by interferon. Proc Natl Acad Sci USA 78(1):129–132. https://doi.org/10.1073/pnas.78.1.129
42. Rodriguez AE, Ducker GS, Billingham LK, Martinez CA, Mainolfi N, Suri V, Friedman A, Manfredi MG, Weinberg SE, Rabinowitz JD, Chandel NS (2019) Serine metabolism supports macrophage IL-1beta production. Cell Metab 29 (4):1003–1011 e1004. https://doi.org/10.1016/j.cmet.2019.01.014
43. Van den Bossche J, Baardman J, de Winther MP (2015) Metabolic Characterization of polarized M1 and M2 bone marrow-derived macrophages using real-time extracellular flux analysis. J Vis Exp (105). https://doi.org/10.3791/53424
44. Huang SC, Everts B, Ivanova Y, O'Sullivan D, Nascimento M, Smith AM, Beatty W, Love-Gregory L, Lam WY, O'Neill CM, Yan C, Du H, Abumrad NA, Urban JF Jr, Artyomov MN, Pearce EL, Pearce EJ (2014) Cell-intrinsic lysosomal lipolysis is essential for alternative activation of macrophages. Nat Immunol 15(9):846–855. https://doi.org/10.1038/ni.2956
45. Xu X, Grijalva A, Skowronski A, van Eijk M, Serlie MJ, Ferrante AW Jr (2013) Obesity activates a program of lysosomal-dependent lipid metabolism in adipose tissue macrophages independently of classic activation. Cell Metab 18(6):816–830. https://doi.org/10.1016/j.cmet.2013.11.001
46. Namgaladze D (1841) Brune B (2014) Fatty acid oxidation is dispensable for human macrophage IL-4-induced polarization. Biochim Biophys Acta 9:1329–1335. https://doi.org/10.1016/j.bbalip.2014.06.007
47. Wang F, Zhang S, Vuckovic I, Jeon R, Lerman A, Folmes CD, Dzeja PP, Herrmann J (2018) Glycolytic stimulation is not a requirement for M2 macrophage differentiation. Cell Metab 28(3):463–475 e464. https://doi.org/10.1016/j.cmet.2018.08.012
48. Langston PK, Shibata M, Horng T (2017) Metabolism supports macrophage activation. Front Immunol 8:61. https://doi.org/10.3389/fimmu.2017.00061
49. Soldano S, Pizzorni C, Paolino S, Trombetta AC, Montagna P, Brizzolara R, Ruaro B, Sulli A, Cutolo M (2017) Correction: alternatively activated (M2) macrophage phenotype is inducible by endothelin-1 in cultured human macrophages. PLoS One 12(3):e0175238. https://doi.org/10.1371/journal.pone.0175238
50. Covarrubias AJ, Aksoylar HI, Yu J, Snyder NW, Worth AJ, Iyer SS, Wang J, Ben-Sahra I, Byles V, Polynne-Stapornkul T, Espinosa EC, Lamming D, Manning BD, Zhang Y, Blair IA, Horng T (2016) Akt-mTORC1 signaling regulates Acly to integrate metabolic input to control of macrophage activation. Elife 5. https://doi.org/10.7554/eLife.11612
51. Powell JD, Pollizzi KN, Heikamp EB, Horton MR (2012) Regulation of immune responses by mTOR. Annu Rev Immunol 30:39–68. https://doi.org/10.1146/annurev-immunol-020711-075024
52. Henin N, Vincent MF, Gruber HE, Van den Berghe G (1995) Inhibition of fatty acid and cholesterol synthesis by stimulation of AMP-activated protein kinase. FASEB J 9(7):541–546. https://doi.org/10.1096/fasebj.9.7.7737463

53. Sag D, Carling D, Stout RD, Suttles J (2008) Adenosine 5'-monophosphate-activated protein kinase promotes macrophage polarization to an anti-inflammatory functional phenotype. J Immunol 181(12):8633–8641. https://doi.org/10.4049/jimmunol.181.12.8633
54. Liu TF, Vachharajani VT, Yoza BK, McCall CE (2012) NAD+-dependent sirtuin 1 and 6 proteins coordinate a switch from glucose to fatty acid oxidation during the acute inflammatory response. J Biol Chem 287(31):25758–25769. https://doi.org/10.1074/jbc.M112.362343
55. Wu G, Morris SM Jr (1998) Arginine metabolism: nitric oxide and beyond. Biochem J 336(Pt 1):1–17. https://doi.org/10.1042/bj3360001
56. Morris SM Jr (2002) Regulation of enzymes of the urea cycle and arginine metabolism. Annu Rev Nutr 22:87–105. https://doi.org/10.1146/annurev.nutr.22.110801.140547
57. Van den Bossche J, Lamers WH, Koehler ES, Geuns JM, Alhonen L, Uimari A, Pirnes-Karhu S, Van Overmeire E, Morias Y, Brys L, Vereecke L, De Baetselier P, Van Ginderachter JA (2012) Pivotal advance: Arginase-1-independent polyamine production stimulates the expression of IL-4-induced alternatively activated macrophage markers while inhibiting LPS-induced expression of inflammatory genes. J Leukoc Biol 91(5):685–699. https://doi.org/10.1189/jlb.0911453
58. Stunault MI, Bories G, Guinamard RR, Ivanov S (2018) Metabolism plays a key role during macrophage activation. Mediators Inflamm 2018:2426138. https://doi.org/10.1155/2018/2426138
59. Baardman J, Licht I, de Winther MP, Van den Bossche J (2015) Metabolic-epigenetic crosstalk in macrophage activation. Epigenomics 7(7):1155–1164. https://doi.org/10.2217/epi.15.71
60. Pietrangelo A, Ouimet M (2019) Death eaters rely on metabolic signaling to wield anti-inflammatory responses. Cell Metab 29(2):234–236. https://doi.org/10.1016/j.cmet.2019.01.010
61. Zhang S, Weinberg S, DeBerge M, Gainullina A, Schipma M, Kinchen JM, Ben-Sahra I, Gius DR, Yvan-Charvet L, Chandel NS, Schumacker PT, Thorp EB (2019) Efferocytosis fuels requirements of fatty acid oxidation and the electron transport chain to polarize macrophages for tissue repair. Cell Metab 29(2):443–456 e445. https://doi.org/10.1016/j.cmet.2018.12.004
62. Park D, Han CZ, Elliott MR, Kinchen JM, Trampont PC, Das S, Collins S, Lysiak JJ, Hoehn KL, Ravichandran KS (2011) Continued clearance of apoptotic cells critically depends on the phagocyte Ucp2 protein. Nature 477(7363):220–224. https://doi.org/10.1038/nature10340
63. Wang Y, Subramanian M, Yurdagul A, Jr., Barbosa-Lorenzi VC, Cai B, de Juan-Sanz J, Ryan TA, Nomura M, Maxfield FR, Tabas I (2017) Mitochondrial fission promotes the continued clearance of apoptotic cells by macrophages. Cell 171(2):331–345 e322. https://doi.org/10.1016/j.cell.2017.08.041
64. Paradisi F, D'Onofrio C, Pepe G, Cifarelli A, Piccolo D (1979) Phagocytosis and cellular metabolism. Ricerca in clinica e in laboratorio 9(1):47–60. https://doi.org/10.1007/bf02905027
65. Cifarelli A, Pepe G, Paradisi F, Piccolo D (1979) The influence of some metabolic inhibitors on phagocytic activity of mouse macrophages in vitro. Res Exp Med 174(2):197–204. https://doi.org/10.1007/bf01851332
66. Artyomov MN, Sergushichev A, Schilling JD (2016) Integrating immunometabolism and macrophage diversity. Semin Immunol 28(5):417–424. https://doi.org/10.1016/j.smim.2016.10.004
67. Svedberg FR, Brown SL, Krauss MZ, Campbell L, Sharpe C, Clausen M, Howell GJ, Clark H, Madsen J, Evans CM, Sutherland TE, Ivens AC, Thornton DJ, Grencis RK, Hussell T, Cunoosamy DM, Cook PC, MacDonald AS (2019) The lung environment controls alveolar macrophage metabolism and responsiveness in type 2 inflammation. Nat Immunol 20(5):571–580. https://doi.org/10.1038/s41590-019-0352-y
68. Izquierdo HM, Brandi P, Gomez MJ, Conde-Garrosa R, Priego E, Enamorado M, Martinez-Cano S, Sanchez I, Conejero L, Jimenez-Carretero D, Martin-Puig S, Guilliams M, Sancho D (2018) Von Hippel-Lindau protein is required for optimal alveolar macrophage terminal differentiation, self-renewal, and function. Cell Rep 24(7):1738–1746. https://doi.org/10.1016/j.celrep.2018.07.034

69. Considine RV (2014) Activated monocytes: yet another link between systemic inflammation and obesity. J Clin Endocrinol Metab 99(7):2347–2349. https://doi.org/10.1210/jc.2014-2095
70. Lachmandas E, Boutens L, Ratter JM, Hijmans A, Hooiveld GJ, Joosten LA, Rodenburg RJ, Fransen JA, Houtkooper RH, van Crevel R, Netea MG, Stienstra R (2016) Microbial stimulation of different Toll-like receptor signalling pathways induces diverse metabolic programmes in human monocytes. Nat Microbiol 2:16246. https://doi.org/10.1038/nmicrobiol.2016.246
71. Fernandez-Ruiz I, Puchalska P, Narasimhulu CA, Sengupta B, Parthasarathy S (2016) Differential lipid metabolism in monocytes and macrophages: influence of cholesterol loading. J Lipid Res 57(4):574–586. https://doi.org/10.1194/jlr.M062752
72. Kramer PA, Ravi S, Chacko B, Johnson MS, Darley-Usmar VM (2014) A review of the mitochondrial and glycolytic metabolism in human platelets and leukocytes: implications for their use as bioenergetic biomarkers. Redox Biol 2:206–210. https://doi.org/10.1016/j.redox.2013.12.026
73. Netea MG, Joosten LAB (2018) Trained immunity and local innate immune memory in the lung. Cell 175(6):1463–1465. https://doi.org/10.1016/j.cell.2018.11.007
74. Arts RJ, Novakovic B, Ter Horst R, Carvalho A, Bekkering S, Lachmandas E, Rodrigues F, Silvestre R, Cheng SC, Wang SY, Habibi E, Goncalves LG, Mesquita I, Cunha C, van Laarhoven A, van de Veerdonk FL, Williams DL, van der Meer JW, Logie C, O'Neill LA, Dinarello CA, Riksen NP, van Crevel R, Clish C, Notebaart RA, Joosten LA, Stunnenberg HG, Xavier RJ, Netea MG (2016) Glutaminolysis and fumarate accumulation integrate immunometabolic and epigenetic programs in trained immunity. Cell Metab 24(6):807–819. https://doi.org/10.1016/j.cmet.2016.10.008
75. Lee HK, Iwasaki A (2007) Innate control of adaptive immunity: dendritic cells and beyond. Semin Immunol 19(1):48–55. https://doi.org/10.1016/j.smim.2006.12.001
76. Audiger C, Rahman MJ, Yun TJ, Tarbell KV, Lesage S (2017) The importance of dendritic cells in maintaining immune tolerance. J Immunol 198(6):2223–2231. https://doi.org/10.4049/jimmunol.1601629
77. Lutz MB, Kukutsch N, Ogilvie AL, Rossner S, Koch F, Romani N, Schuler G (1999) An advanced culture method for generating large quantities of highly pure dendritic cells from mouse bone marrow. J Immunol Methods 223(1):77–92
78. Krawczyk CM, Holowka T, Sun J, Blagih J, Amiel E, DeBerardinis RJ, Cross JR, Jung E, Thompson CB, Jones RG, Pearce EJ (2010) Toll-like receptor-induced changes in glycolytic metabolism regulate dendritic cell activation. Blood 115(23):4742–4749. https://doi.org/10.1182/blood-2009-10-249540
79. Xu X, Ye L, Araki K, Ahmed R (2012) mTOR, linking metabolism and immunity. Semin Immunol 24(6):429–435. https://doi.org/10.1016/j.smim.2012.12.005
80. Freemerman AJ, Zhao L, Pingili AK, Teng B, Cozzo AJ, Fuller AM, Johnson AR, Milner JJ, Lim MF, Galanko JA, Beck MA, Bear JE, Rotty JD, Bezavada L, Smallwood HS, Puchowicz MA, Liu J, Locasale JW, Lee DP, Bennett BJ, Abel ED, Rathmell JC, Makowski L (2019) Myeloid Slc2a1-deficient murine model revealed macrophage activation and metabolic phenotype are fueled by GLUT1. J Immunol 202(4):1265–1286. https://doi.org/10.4049/jimmunol.1800002
81. Thwe PM, Pelgrom LR, Cooper R, Beauchamp S, Reisz JA, D'Alessandro A, Everts B, Amiel E (2017) Cell-intrinsic glycogen metabolism supports early glycolytic reprogramming required for dendritic cell immune responses. Cell Metab 26(3):558–567 e555. https://doi.org/10.1016/j.cmet.2017.08.012
82. Braun A, Worbs T, Moschovakis GL, Halle S, Hoffmann K, Bolter J, Munk A, Forster R (2011) Afferent lymph-derived T cells and DCs use different chemokine receptor CCR7-dependent routes for entry into the lymph node and intranodal migration. Nat Immunol 12(9):879–887. https://doi.org/10.1038/ni.2085
83. Guak H, Al Habyan S, Ma EH, Aldossary H, Al-Masri M, Won SY, Ying T, Fixman ED, Jones RG, McCaffrey LM, Krawczyk CM (2018) Glycolytic metabolism is essential for CCR7 oligomerization and dendritic cell migration. Nat Commun 9(1):2463. https://doi.org/10.1038/s41467-018-04804-6

84. Cleeter MW, Cooper JM, Darley-Usmar VM, Moncada S, Schapira AH (1994) Reversible inhibition of cytochrome c oxidase, the terminal enzyme of the mitochondrial respiratory chain, by nitric oxide. Implications for neurodegenerative diseases. FEBS Lett 345(1):50–54. https://doi.org/10.1016/0014-5793(94)00424-2
85. Everts B, Amiel E, van der Windt GJ, Freitas TC, Chott R, Yarasheski KE, Pearce EL, Pearce EJ (2012) Commitment to glycolysis sustains survival of NO-producing inflammatory dendritic cells. Blood 120(7):1422–1431. https://doi.org/10.1182/blood-2012-03-419747
86. Lawless SJ, Kedia-Mehta N, Walls JF, McGarrigle R, Convery O, Sinclair LV, Navarro MN, Murray J, Finlay DK (2017) Glucose represses dendritic cell-induced T cell responses. Nat Commun 8:15620. https://doi.org/10.1038/ncomms15620
87. Gotoh K, Morisaki T, Setoyama D, Sasaki K, Yagi M, Igami K, Mizuguchi S, Uchiumi T, Fukui Y, Kang D (2018) Mitochondrial p32/C1qbp is a critical regulator of dendritic cell metabolism and maturation. Cell Rep 25(7):1800–1815 e1804. https://doi.org/10.1016/j.celrep.2018.10.057
88. Hoepel W, Newling M, Vogelpoel LTC, Sritharan L, Hansen IS, Kapsenberg ML, Baeten DLP, Everts B, den Dunnen J (2019) FcgammaR-TLR cross-talk enhances TNF production by human monocyte-derived DCs via IRF5-dependent gene transcription and glycolytic reprogramming. Front Immunol 10:739. https://doi.org/10.3389/fimmu.2019.00739
89. Rubic T, Lametschwandtner G, Jost S, Hinteregger S, Kund J, Carballido-Perrig N, Schwarzler C, Junt T, Voshol H, Meingassner JG, Mao X, Werner G, Rot A, Carballido JM (2008) Triggering the succinate receptor GPR91 on dendritic cells enhances immunity. Nat Immunol 9(11):1261–1269. https://doi.org/10.1038/ni.1657
90. Pantel A, Teixeira A, Haddad E, Wood EG, Steinman RM, Longhi MP (2014) Direct type I IFN but not MDA5/TLR3 activation of dendritic cells is required for maturation and metabolic shift to glycolysis after poly IC stimulation. PLoS Biol 12(1):e1001759. https://doi.org/10.1371/journal.pbio.1001759
91. Turnquist HR, Raimondi G, Zahorchak AF, Fischer RT, Wang Z, Thomson AW (2007) Rapamycin-conditioned dendritic cells are poor stimulators of allogeneic CD4+ T cells, but enrich for antigen-specific Foxp3+ T regulatory cells and promote organ transplant tolerance. J Immunol 178(11):7018–7031. https://doi.org/10.4049/jimmunol.178.11.7018
92. Amiel E, Everts B, Freitas TC, King IL, Curtis JD, Pearce EL, Pearce EJ (2012) Inhibition of mechanistic target of rapamycin promotes dendritic cell activation and enhances therapeutic autologous vaccination in mice. J Immunol 189(5):2151–2158. https://doi.org/10.4049/jimmunol.1103741
93. Procaccini C, De Rosa V, Galgani M, Carbone F, Cassano S, Greco D, Qian K, Auvinen P, Cali G, Stallone G, Formisano L, La Cava A, Matarese G (2012) Leptin-induced mTOR activation defines a specific molecular and transcriptional signature controlling CD4+ effector T cell responses. J Immunol 189(6):2941–2953. https://doi.org/10.4049/jimmunol.1200935
94. Moraes-Vieira PM, Larocca RA, Bassi EJ, Peron JP, Andrade-Oliveira V, Wasinski F, Araujo R, Thornley T, Quintana FJ, Basso AS, Strom TB, Camara NO (2014) Leptin deficiency impairs maturation of dendritic cells and enhances induction of regulatory T and Th17 cells. Eur J Immunol 44(3):794–806. https://doi.org/10.1002/eji.201343592
95. Perrin-Cocon L, Aublin-Gex A, Diaz O, Ramiere C, Peri F, Andre P, Lotteau V (2018) Toll-like receptor 4-induced glycolytic burst in human monocyte-derived dendritic cells results from p38-dependent stabilization of HIF-1alpha and increased Hexokinase II expression. J Immunol 201(5):1510–1521. https://doi.org/10.4049/jimmunol.1701522
96. Carroll KC, Viollet B, Suttles J (2013) AMPKalpha1 deficiency amplifies proinflammatory myeloid APC activity and CD40 signaling. J Leukoc Biol 94(6):1113–1121. https://doi.org/10.1189/jlb.0313157
97. Ito T, Wang YH, Liu YJ (2005) Plasmacytoid dendritic cell precursors/type I interferon-producing cells sense viral infection by Toll-like receptor (TLR) 7 and TLR9. Springer Semin Immunopathol 26(3):221–229. https://doi.org/10.1007/s00281-004-0180-4
98. Cao W, Manicassamy S, Tang H, Kasturi SP, Pirani A, Murthy N, Pulendran B (2008) Toll-like receptor-mediated induction of type I interferon in plasmacytoid dendritic cells requires

the rapamycin-sensitive PI(3)K-mTOR-p70S6K pathway. Nat Immunol 9(10):1157–1164. https://doi.org/10.1038/ni.1645
99. Bajwa G, DeBerardinis RJ, Shao B, Hall B, Farrar JD, Gill MA (2016) Cutting edge: critical role of glycolysis in human plasmacytoid dendritic cell antiviral responses. J Immunol 196(5):2004–2009. https://doi.org/10.4049/jimmunol.1501557
100. Banchereau J, Pascual V (2006) Type I interferon in systemic lupus erythematosus and other autoimmune diseases. Immunity 25(3):383–392. https://doi.org/10.1016/j.immuni.2006.08.010
101. Wu D, Sanin DE, Everts B, Chen Q, Qiu J, Buck MD, Patterson A, Smith AM, Chang CH, Liu Z, Artyomov MN, Pearce EL, Cella M, Pearce EJ (2016) Type 1 interferons induce changes in core metabolism that are critical for immune function. Immunity 44(6):1325–1336. https://doi.org/10.1016/j.immuni.2016.06.006
102. Morelli AE, Thomson AW (2007) Tolerogenic dendritic cells and the quest for transplant tolerance. Nat Rev Immunol 7(8):610–621. https://doi.org/10.1038/nri2132
103. Malinarich F, Duan K, Hamid RA, Bijin A, Lin WX, Poidinger M, Fairhurst AM, Connolly JE (2015) High mitochondrial respiration and glycolytic capacity represent a metabolic phenotype of human tolerogenic dendritic cells. J Immunol 194(11):5174–5186. https://doi.org/10.4049/jimmunol.1303316
104. Ferreira GB, Vanherwegen AS, Eelen G, Gutierrez ACF, Van Lommel L, Marchal K, Verlinden L, Verstuyf A, Nogueira T, Georgiadou M, Schuit F, Eizirik DL, Gysemans C, Carmeliet P, Overbergh L, Mathieu C (2015) Vitamin D3 induces tolerance in human dendritic cells by activation of intracellular metabolic pathways. Cell Rep 10(5):711–725. https://doi.org/10.1016/j.celrep.2015.01.013
105. Mondanelli G, Bianchi R, Pallotta MT, Orabona C, Albini E, Iacono A, Belladonna ML, Vacca C, Fallarino F, Macchiarulo A, Ugel S, Bronte V, Gevi F, Zolla L, Verhaar A, Peppelenbosch M, Mazza EMC, Bicciato S, Laouar Y, Santambrogio L, Puccetti P, Volpi C, Grohmann U (2017) A Relay Pathway between arginine and tryptophan metabolism confers immunosuppressive properties on dendritic cells. Immunity 46(2):233–244. https://doi.org/10.1016/j.immuni.2017.01.005
106. Pelgrom LR, Patente TA, Sergushichev A, Esaulova E, Otto F, Ozir-Fazalalikhan A, van der Zande HJP, van der Ham AJ, van der Stel S, Artyomov MN, Everts B (2019) LKB1 expressed in dendritic cells governs the development and expansion of thymus-derived regulatory T cells. Cell Res 29(5):406–419. https://doi.org/10.1038/s41422-019-0161-8
107. Zheng X, Chen M, Meng X, Chu X, Cai C, Zou F (2017) Phosphorylation of dynamin-related protein 1 at Ser616 regulates mitochondrial fission and is involved in mitochondrial calcium uniporter-mediated neutrophil polarization and chemotaxis. Mol Immunol 87:23–32. https://doi.org/10.1016/j.molimm.2017.03.019
108. Bao Y, Ledderose C, Graf AF, Brix B, Birsak T, Lee A, Zhang J, Junger WG (2015) mTOR and differential activation of mitochondria orchestrate neutrophil chemotaxis. J Cell Biol 210(7):1153–1164. https://doi.org/10.1083/jcb.201503066
109. Rodriguez-Espinosa O, Rojas-Espinosa O, Moreno-Altamirano MM, Lopez-Villegas EO, Sanchez-Garcia FJ (2015) Metabolic requirements for neutrophil extracellular traps formation. Immunology 145(2):213–224. https://doi.org/10.1111/imm.12437
110. Douda DN, Khan MA, Grasemann H, Palaniyar N (2015) SK3 channel and mitochondrial ROS mediate NADPH oxidase-independent NETosis induced by calcium influx. Proc Natl Acad Sci USA 112(9):2817–2822. https://doi.org/10.1073/pnas.1414055112
111. Bao Y, Ledderose C, Seier T, Graf AF, Brix B, Chong E, Junger WG (2014) Mitochondria regulate neutrophil activation by generating ATP for autocrine purinergic signaling. J Biol Chem 289(39):26794–26803. https://doi.org/10.1074/jbc.M114.572495
112. Masuda M, Horisaka K, Koeda T (1984) Influences of taurine on functions of rat neutrophils. Jpn J Pharmacol 34(1):116–118
113. Richer BC, Salei N, Laskay T, Seeger K (2018) Changes in Neutrophil Metabolism upon Activation and Aging. Inflammation 41(2):710–721. https://doi.org/10.1007/s10753-017-0725-z

114. Fossati G, Moulding DA, Spiller DG, Moots RJ, White MR, Edwards SW (2003) The mitochondrial network of human neutrophils: role in chemotaxis, phagocytosis, respiratory burst activation, and commitment to apoptosis. J Immunol 170(4):1964–1972. https://doi.org/10.4049/jimmunol.170.4.1964
115. Maianski NA, Geissler J, Srinivasula SM, Alnemri ES, Roos D, Kuijpers TW (2004) Functional characterization of mitochondria in neutrophils: a role restricted to apoptosis. Cell Death Differ 11(2):143–153. https://doi.org/10.1038/sj.cdd.4401320
116. Humbles AA, Lloyd CM, McMillan SJ, Friend DS, Xanthou G, McKenna EE, Ghiran S, Gerard NP, Yu C, Orkin SH, Gerard C (2004) A critical role for eosinophils in allergic airways remodeling. Science 305(5691):1776–1779. https://doi.org/10.1126/science.1100283
117. Fulkerson PC, Rothenberg ME (2013) Targeting eosinophils in allergy, inflammation and beyond. Nat Rev Drug Discov 12(2):117–129. https://doi.org/10.1038/nrd3838
118. Shen ZJ, Malter JS (2015) Determinants of eosinophil survival and apoptotic cell death. Apoptosis 20(2):224–234. https://doi.org/10.1007/s10495-014-1072-2
119. Peachman KK, Lyles DS, Bass DA (2001) Mitochondria in eosinophils: functional role in apoptosis but not respiration. Proc Natl Acad Sci USA 98(4):1717–1722. https://doi.org/10.1073/pnas.98.4.1717
120. Porter L, Toepfner N, Bashant KR, Guck J, Ashcroft M, Farahi N, Chilvers ER (2018) Metabolic profiling of human eosinophils. Front Immunol 9:1404. https://doi.org/10.3389/fimmu.2018.01404
121. DeChatelet LR, Shirley PS, McPhail LC, Huntley CC, Muss HB, Bass DA (1977) Oxidative metabolism of the human eosinophil. Blood 50(3):525–535
122. Voehringer D (2013) Protective and pathological roles of mast cells and basophils. Nat Rev Immunol 13(5):362–375. https://doi.org/10.1038/nri3427
123. Galli SJ, Tsai M (2012) IgE and mast cells in allergic disease. Nat Med 18(5):693–704. https://doi.org/10.1038/nm.2755
124. Phong B, Avery L, Menk AV, Delgoffe GM, Kane LP (2017) Cutting edge: murine mast cells rapidly modulate metabolic pathways essential for distinct effector functions. J Immunol 198(2):640–644. https://doi.org/10.4049/jimmunol.1601150
125. Caslin HL, Abebayehu D, Abdul Qayum A, Haque TT, Taruselli MT, Paez PA, Pondicherry N, Barnstein BO, Hoeferlin LA, Chalfant CE, Ryan JJ (2019) Lactic acid inhibits lipopolysaccharide-induced mast cell function by limiting glycolysis and ATP availability. J Immunol. https://doi.org/10.4049/jimmunol.1801005
126. Saluja R, Khan M, Church MK, Maurer M (2015) The role of IL-33 and mast cells in allergy and inflammation. Clin Transl Allergy 5:33. https://doi.org/10.1186/s13601-015-0076-5
127. Caslin HL, Taruselli MT, Haque T, Pondicherry N, Baldwin EA, Barnstein BO, Ryan JJ (2018) Inhibiting glycolysis and ATP production attenuates IL-33-mediated mast cell function and peritonitis. Front Immunol 9:3026. https://doi.org/10.3389/fimmu.2018.03026
128. Erlich TH, Yagil Z, Kay G, Peretz A, Migalovich-Sheikhet H, Tshori S, Nechushtan H, Levi-Schaffer F, Saada A, Razin E (2014) Mitochondrial STAT3 plays a major role in IgE-antigen-mediated mast cell exocytosis. J Allergy Clin Immunol 134(2):460–469. https://doi.org/10.1016/j.jaci.2013.12.1075
129. Chmelar J, Chatzigeorgiou A, Chung KJ, Prucnal M, Voehringer D, Roers A, Chavakis T (2016) No role for mast cells in obesity-related metabolic dysregulation. Front Immunol 7:524. https://doi.org/10.3389/fimmu.2016.00524
130. Mah AY, Cooper MA (2016) Metabolic regulation of natural killer cell IFN-gamma production. Crit Rev Immunol 36(2):131–147. https://doi.org/10.1615/CritRevImmunol.2016017387
131. Wang R, Jaw JJ, Stutzman NC, Zou Z, Sun PD (2012) Natural killer cell-produced IFN-gamma and TNF-alpha induce target cell cytolysis through up-regulation of ICAM-1. J Leukoc Biol 91(2):299–309. https://doi.org/10.1189/jlb.0611308
132. Keppel MP, Saucier N, Mah AY, Vogel TP, Cooper MA (2015) Activation-specific metabolic requirements for NK Cell IFN-gamma production. J Immunol 194(4):1954–1962. https://doi.org/10.4049/jimmunol.1402099

133. Marcais A, Cherfils-Vicini J, Viant C, Degouve S, Viel S, Fenis A, Rabilloud J, Mayol K, Tavares A, Bienvenu J, Gangloff YG, Gilson E, Vivier E, Walzer T (2014) The metabolic checkpoint kinase mTOR is essential for IL-15 signaling during the development and activation of NK cells. Nat Immunol 15(8):749–757. https://doi.org/10.1038/ni.2936
134. Donnelly RP, Loftus RM, Keating SE, Liou KT, Biron CA, Gardiner CM, Finlay DK (2014) mTORC1-dependent metabolic reprogramming is a prerequisite for NK cell effector function. J Immunol 193(9):4477–4484. https://doi.org/10.4049/jimmunol.1401558
135. Loftus RM, Finlay DK (2016) Immunometabolism: cellular metabolism turns immune regulator. J Biol Chem 291(1):1–10. https://doi.org/10.1074/jbc.R115.693903
136. Mao Y, van Hoef V, Zhang X, Wennerberg E, Lorent J, Witt K, Masvidal L, Liang S, Murray S, Larsson O, Kiessling R, Lundqvist A (2016) IL-15 activates mTOR and primes stress-activated gene expression leading to prolonged antitumor capacity of NK cells. Blood 128(11):1475–1489. https://doi.org/10.1182/blood-2016-02-698027
137. Keating SE, Zaiatz-Bittencourt V, Loftus RM, Keane C, Brennan K, Finlay DK, Gardiner CM (2016) Metabolic reprogramming supports IFN-gamma production by CD56bright NK cells. J Immunol 196(6):2552–2560. https://doi.org/10.4049/jimmunol.1501783
138. Spits H, Artis D, Colonna M, Diefenbach A, Di Santo JP, Eberl G, Koyasu S, Locksley RM, McKenzie AN, Mebius RE, Powrie F, Vivier E (2013) Innate lymphoid cells–a proposal for uniform nomenclature. Nat Rev Immunol 13(2):145–149. https://doi.org/10.1038/nri3365
139. Monticelli LA, Buck MD, Flamar AL, Saenz SA, Tait Wojno ED, Yudanin NA, Osborne LC, Hepworth MR, Tran SV, Rodewald HR, Shah H, Cross JR, Diamond JM, Cantu E, Christie JD, Pearce EL, Artis D (2016) Arginase 1 is an innate lymphoid-cell-intrinsic metabolic checkpoint controlling type 2 inflammation. Nat Immunol 17(6):656–665. https://doi.org/10.1038/ni.3421
140. Bando JK, Nussbaum JC, Liang HE, Locksley RM (2013) Type 2 innate lymphoid cells constitutively express arginase-I in the naive and inflamed lung. J Leukoc Biol 94(5):877–884. https://doi.org/10.1189/jlb.0213084
141. Li Q, Li D, Zhang X, Wan Q, Zhang W, Zheng M, Zou L, Elly C, Lee JH, Liu YC (2018) E3 Ligase VHL Promotes group 2 innate lymphoid cell maturation and function via glycolysis inhibition and induction of interleukin-33 receptor. Immunity 48(2):258–270 e255. https://doi.org/10.1016/j.immuni.2017.12.013
142. Brestoff JR, Kim BS, Saenz SA, Stine RR, Monticelli LA, Sonnenberg GF, Thome JJ, Farber DL, Lutfy K, Seale P, Artis D (2015) Group 2 innate lymphoid cells promote beiging of white adipose tissue and limit obesity. Nature 519(7542):242–246. https://doi.org/10.1038/nature14115
143. Wilhelm C, Harrison OJ, Schmitt V, Pelletier M, Spencer SP, Urban JF Jr, Ploch M, Ramalingam TR, Siegel RM, Belkaid Y (2016) Critical role of fatty acid metabolism in ILC2-mediated barrier protection during malnutrition and helminth infection. J Exp Med 213(8):1409–1418. https://doi.org/10.1084/jem.20151448
144. Gury-BenAri M, Thaiss CA, Serafini N, Winter DR, Giladi A, Lara-Astiaso D, Levy M, Salame TM, Weiner A, David E, Shapiro H, Dori-Bachash M, Pevsner-Fischer M, Lorenzo-Vivas E, Keren-Shaul H, Paul F, Harmelin A, Eberl G, Itzkovitz S, Tanay A, Di Santo JP, Elinav E, Amit I (2016) The spectrum and regulatory landscape of intestinal innate lymphoid cells are shaped by the microbiome. Cell 166 (5):1231–1246 e1213. https://doi.org/10.1016/j.cell.2016.07.043
145. Pearce EL, Pearce EJ (2013) Metabolic pathways in immune cell activation and quiescence. Immunity 38(4):633–643. https://doi.org/10.1016/j.immuni.2013.04.005
146. Di Luccia B, Gilfillan S, Cella M, Colonna M, Huang SC (2019) ILC3s integrate glycolysis and mitochondrial production of reactive oxygen species to fulfill activation demands. J Exp Med. https://doi.org/10.1084/jem.20180549

Metabolic Profile of Adaptive Immune Cells

Omar Domínguez-Amorocho, Gonzalo Soto-Heredero, and María Mittelbrunn

1 Introduction

Metabolism is the core process for biological systems required for energy provision and for macromolecules synthesis. The influence of metabolism in the immune system has been recognized for the last century, as malnutrition led to increase susceptibility to infection or obesity is associated with autoimmunity. The immune system has a wide repertoire of cells with specific functions and demands. Each cell type or even the same cell at different states have distinct anabolic and catabolic demands that can be fulfilled by processing specific substrates. Different studies have showed that cellular metabolism plays an important role on the fate and function of the immune cells. Thus, modulating the immune response by controlling the metabolism of the immune cells have immense therapeutics applications for inflammatory and autoimmune diseases, cancer, infection diseases and even age-related disorders [26, 31, 38, 68, 83, 88].

O. Domínguez-Amorocho · G. Soto-Heredero · M. Mittelbrunn (✉)
Immunometabolism and Inflammation Laboratory, Centro de Biología Molecular Severo Ochoa, Cellular Communication & Inflammation Unit, Madrid, Spain
e-mail: mmittelbrunn@cbm.csic.es

Faculty of Sciences, Department of Molecular Biology, Universidad Autónoma de Madrid (UAM), Madrid, Spain

O. Domínguez-Amorocho
e-mail: o.dominguez@usp.br

G. Soto-Heredero
e-mail: gonzalo.soto@cbm.csic.es

O. Domínguez-Amorocho
Institute of Biomedical Sciences, University of São Paulo, São Paulo, Brazil

G. Soto-Heredero · M. Mittelbrunn
Instituto de Investigación Sanitaria Hospital, 12 de Octubre (imas12), Madrid, Spain

N. O. S. Camara et al. (eds.), *Essential Aspects of Immunometabolism in Health and Disease*, https://doi.org/10.1007/978-3-030-86684-6_6

Innate and adaptive branches of the immune system can be modulated by metabolism. The adaptive immunity provides the immune system with the capacity to recognize specific pathogens and other structures, generating specific immunity and memory. Adaptive immune responses are mediated by two specific compartments of the lymphoid lineage, T and B lymphocytes, which display different functional properties depending on their development and differentiation.

Adaptive immune responses require a balance between development, maintenance and tuning mediated by different profiles of effector, regulatory and memory T cells and the maturation of B cells. Each cell subset presents a distinct metabolic program depending on their activation level, and nutrient adaptations or metabolic reprogramming are closely related to the acquisition or repression of different effector or regulation functions. Metabolites derived from these metabolic conditions determine the final activation state and function of the immune cells.

Cell metabolism depends on uptake, processing and use of different nutrients, mainly glucose, amino acids, and fatty acids (FA) and some of their sub products. The availability of the different nutrients depends on the tissue composition or transient conditions of the microenvironment, suggesting that the regulation of nutrient availability through competitive uptake is an important regulation point in the control of metabolism [56]. In addition, some factors like microbiota and organs as liver, pancreas and intestine can regulate the nutrients or metabolites availability, having a final effect on the immune response [95]. The main metabolic pathways related to the behavior of immune cells are glycolysis, tricarboxylic acid (TCA) cycle, fatty acid oxidation (FAO), fatty acid synthesis (FAS), pentose phosphate pathway (PPP), and amino acid synthesis [82, 89]. Other associated pathways have been showed as immune response modulators, mainly those related to the biosynthesis of polyamines, cholesterol, hexosamines, and nucleotides [94, 95].

Lymphoid cells predominate in a naïve state with a quiescent metabolism that mainly depends on OXPHOS to obtain ATP. When an antigen is recognized, lymphocytes switch their metabolic program to fulfil higher energetic demand. Therefore, a metabolic reprogramming with enhanced glycolytic rate is needed to ensure rapid growth, proliferation and differentiation [118]. Once the pathogenic agent has been eliminated, immune cells switch once more their metabolism towards OXPHOS, acquiring a pro-resolving phenotype [105]. In this chapter, we discuss metabolic modulation of the adaptive immune response and summarize the metabolic changes that define adaptive immune cells functions and the molecular drivers of that metabolic shift.

2 Cellular Metabolism in T Cells

T cells are one of the main elements of the adaptive immunity and are comprised by two main subsets with diverse phenotypical plasticity and function, the CD4 and CD8 T cells. Upon antigen recognition, T cells experience substantial modifications in their metabolism, a process called “metabolic reprogramming”, to ensure energy

availability for clonal expansion and production of effector molecules [78]. Here, we will review the metabolic profile of T cells from their generation in the thymus, followed by their activation, differentiation and finally their exhaustion.

2.1 T Cell Metabolism During Ontogeny and Activation

T cells precursors are generated in the bone marrow and migrate to the thymus, to complete their development and functional selection. Early thymocyte development in the thymus is coordinated by two main extracellular signals, Notch1 and IL-7 [99]. Notch1 signaling stimulates glucose and glutamine metabolism and mediate catalytic reactions important for self-renewal of TCR-β-selected progenitors [54]. On the other side, IL-7 promotes the growth and survival of early thymocytes, likely through mTORC1 signaling activation, increasing glycolysis and oxygen consumption during early developmental stages [54, 125].

Once naïve T cells have been selected, they exit the thymus and circulate the periphery as metabolically quiescent cells that require minimal nutrient uptake and metabolic demands for survival. In this resting conditions, naïve immune cells rely preferentially on OXPHOS to fulfil their low energetic demands [44]. Upon activation, T cells increase their size and proliferation rate, and they need to adapt their metabolism. Despite glycolysis is much less effective than OXPHOS for ATP production, it is faster and is the preferred metabolism of highly proliferating cells like effector T cells.

Upon early T cell activation, TCR signaling activates mTORC1 which supports the expression of enzymes participating in glycolysis and glutaminolysis, shifting the cellular metabolism [51, 120, 126]. In addition to glycolysis and glutaminolysis, the PPP and the hexosamine pathway are also enhanced in T cells upon activation [105]. The metabolic reprogramming induced by mTORC1 exits T cell from quiescence and is essential for CD4 and CD8 T cell development, homeostasis, differentiation and function [19, 24, 80, 91, 92, 103, 126]. This metabolic switch is mediated by the activation of the transcription factors c-Myc and HIF-1α. HIF-1α enhances glycolysis and PPP, promoting ATP production and increasing biosynthetic capacity of the cell [30, 109]. One of the targets of HIF-1α is Glut1, which is upregulated, increasing the glucose uptake. Glut1 deficiency prevents the increase of glucose uptake and glycolysis, decreasing effector T cells survival and differentiation [72].

In addition, HIF-1α activates the pyruvate dehydrogenase kinase 1 (PDK1), an enzyme that avoids pyruvate entering the TCA cycle [39, 75], promoting the conversion of pyruvate to lactate and thus enhancing glycolysis [35, 40, 89].

On the other hand, c-Myc also contributes to increase the glycolytic rate [35, 72, 120]. Deletion of c-Myc blocks both glycolysis and glutaminolysis, impairing the activation and proliferation in culturing T cells [120].

Although T cells increase their glycolytic flux during activation, they also require functional mitochondrial to support their highly proliferation rate. Signals

coming from the mitochondria, such as reactive oxygen species (ROS) generated by mitochondrial complex III are required for optimal proliferation rate [108].

As mentioned, catabolism of glutamine (or glutaminolysis) is also an important metabolic pathway for T cell activation. Glutaminolysis products go through the TCA cycle for the de novo synthesis of lipids and fuels redox and epigenetic reactions. The glutaminase catalyzes the first reaction of glutaminolysis which is the transformation of glutamine into glutamate. Lack of glutaminase has been connected with reduced T cell activation and proliferation proving an important role of glutamine metabolism in T cell function [51].

Upon activation, T cells need amino acids metabolism to obtain specific intermediates that are essential for T cell function. Amino acids uptake is mediated by the L-type amino acid transporter 1 (LAT1), which is upregulated in human T cells upon CD3/CD28 stimulation. Functional disturbance of LAT1 in T cells affects the uptake of essential amino acids, activating the DNA damage-inducible transcript 3, a DNA-mediated stress response that inhibits NF-κB and NFAT signaling and reduces cytokine production [43]. The metabolism of serine and glycine seems to be indispensable for T cell activation since activated T cells upregulate enzymes of the serine and glycine metabolism. Serine is necessary for optimal T cell expansion even in glucose availability conditions. The metabolism of serine supplies the necessary glycine for de novo biosynthesis in proliferating T cells, indicating that serine is a crucial immunometabolite for T cells proliferative capacity [71]. Extracellular alanine is also needed for effective transit from quiescence to activation. Alanine deprivation impairs both metabolism and function of activated T cell, reducing IL-17, IFN-γ and IL-6 secretion [97].

FA are crucial resources for cellular energy and for generation of complex lipids such as cholesterol and membrane phospholipids. Accordingly, the abundance of FA and the signaling by intracellular lipids play a key role in T cell development [3, 46, 81]. Initiation of de novo synthesis of FA is critical for proliferation and differentiation of activated effector T cells [67]. In addition, FA offer intermediates for the TCA cycle, important for $CD8^+$ T cell survival and clonal expansion [64].

2.2 T Cell Metabolism During Functional Differentiation

Upon stimulation, $CD4^+$ T lymphocytes can be differentiated into diverse effector T subpopulations, which are pro-inflammatory cells, or into regulatory T cells (Treg). The glucose metabolism is essential for Th1, Th2, and Th17 effector subsets differentiation. Consequently, the glycolysis initiation is a lineage-decisive event for the differentiation of these effector subpopulations, which display a strong bias towards glycolysis over mitochondrial metabolism [17, 95]. mTORC1 activation promotes polarization towards Th1 and Th17 subset and inhibits Treg differentiation [22, 61, 109]. Hence, inhibition of mTORC1 prevents Th1 polarization [25], and inhibition of mTORC2 avoids Th1 and Th2 differentiation [25, 65]. Although mTORC1 deficient $CD4^+$ T cells present impaired differentiation, they present some

memory-related attributes such as surface marker expression, lower metabolic rate and increased longevity [53]. In addition, the PI3K-Akt-mTORC1 axis influences activation, survival, and proliferation of $CD4^+$ T cells after stimulation. Suppression of this axis impairs Th17 polarization by inhibiting the translocation of RORγt to the nucleus [61]. HIF-1α increases glycolysis promoting the polarization of $CD4^+$ T cells to the Th17 subsets by direct activation of the transcription factor RORgt, and simultaneously inhibits the differentiation of Tregs in vitro by direct repression of the transcription factor FoxP3 [10, 22, 109].

Regarding $CD8^+$ T cells, while mTORC1 influences effector responses, mTORC2 activity regulates the formation of memory cells, linking the cellular metabolism to the effector function and memory generation [91]. HIF-1α is necessary for $CD8^+$ T cells cytolytic function and memory generation [35].

Evidence has shown that glutaminolysis is also relevant for $CD4^+$ T cell differentiation. Glutaminase deficiency impairs Th17 differentiation, suggesting an important role of glutaminolysis for Th17 subset [51]. In vivo stimulation of naïve $CD4^+$ T cells in conditions of glutamine restriction results in biased differentiation to functional Treg [60, 79]. Metabolites resulting from glutaminolysis can module the differentiation as well. The α-ketoglutarate (αKG) is a glutamine-derived metabolite that can be incorporated into the TCA cycle. Moreover, αKG plays a role as a metabolic regulator of $CD4^+$ T cell differentiation by promoting Th1 polarization. Certainly, αKG activates mTORC1 signaling and induces the expression of the Th1 transcription factor T-bet, suggesting that glutamine availability controls the equilibrium between the Th1 and Treg cells [60].

Some findings also indicate that certain lipids such as cholesterol and FA are crucial components of metabolic control specially in the functional differentiation of Th17 cells. Specific lipids can be metabolized into lipidic ligands of RORγ, controlling its transcriptional activity [7, 11, 23, 57].

2.3 T Cell Metabolism During Migration

mTORC1 and HIF-1α have a decisive role in the migration of T cells. In addition to promote the expression of glucose transporters and glycolytic enzymes, the mTORC1/HIF-1α axis rules the T cell trafficking by promoting chemokines production and adhesion receptors expression. The mTORC1/HIF-1α axis stimulates the delivery of T cells to the blood by the inhibition of the adhesion molecule CD62L and chemokine receptor CCR7 [35, 77]. mTORC2 is also required for Tregs migration [59]. Interestingly, migratory signals may alter the T cell metabolism by enhancing glucokinase expression and causing rearrangements in the actin cytoskeleton in Tregs [58].

2.4 T Cell Metabolism in the Contraction Phase

When the inflammatory process is over, T cells acquire a pro-resolving phenotype to gradually get back to a homeostatic state. Tregs and memory T cells generation are the more important processes in the resolution phase. In contrast to effector T cells, Tregs and memory T cells rely on OXPHOS and FAO [15, 84]. Consequently, impairment of OXPHOS specifically in Tregs by deletion of mitochondrial complex III produces an autoimmune syndrome and premature death [37, 122]. Conversely, a closer look into Tregs shows a mixed metabolic signature involving OXPHOS, FAO but also glycolysis [95]. Strikingly, it seems that FA are de novo synthesized instead of up taken in memory T cells [84].

FA catabolism via β-oxidation is essential for the differentiation of memory T cells and Tregs [67, 117]. Furthermore, increasing FAO after stimulation boosts the development of memory T cells [90]. In addition, cytokines that triggers memory phenotype in T cells, for example IL-15, promotes expression of FAO enzymes and activates mitochondrial biogenesis [116], supporting that FAO is the principal source of energy of memory T cells. Although they rely on mitochondrial metabolism, memory T cells induce the glycolytic switch faster than naïve T cells [42, 117].

The generation of Tregs and memory T cells is mediated by Bcl-6, a transcription factor that antagonizes the c-Myc and HIF-1α metabolic program and drives a pro-resolving metabolism. Once the damaging agent has been neutralized, the pro-inflammatory cytokine IL-2 is decreased. When IL-2 signal is low, Bcl-6 is activated and shuts down glycolysis in T cells [85]. AMPK and TRAF6 signaling pathways also contribute to maintain this distinguishing metabolic signature of Tregs and memory T cells biased towards OXPHOS and FAO [76, 90, 96]. AMPK activation mediates the increase in FAO and inhibits mTOR, causing a reduction of glucose uptake and glycolysis, favoring Tregs generation [25, 76].

Due to mTOR is needed for effector $CD4^+$ and $CD8^+$ activation, it has been postulated as negative regulator of Tregs. However, since Tregs present higher basal levels of mTORC1 activation, mTOR must drive transcriptional and metabolic programs in activated Tregs [93, 128]. Actually, specific deletion of mTOR in Tregs provokes an autoimmune disease and premature death due to excessive stimulation of effector T cells, suggesting mTOR is essential for Tregs [101, 128]. Recently, it has been shown that the contribution of mTOR to Tregs function is through the control of the mitochondrial metabolism since mitochondrial impairment in Tregs causes the same autoimmune disorder that mTOR deletion [16]. In this context, the interferon regulatory factor 4 (IRF4), which is activated by mTOR, is necessary for glycolytic and nucleotide metabolism upregulation during Treg activation since IRF4 promotes Tregs function and homeostasis [16, 66].

Like mTOR, c-Myc works as an important factor in Treg activation and expansion, even though it is a glycolytic factor. Specific deletion of c-Myc in Tregs triggers an autoimmune syndrome portrayed by uncontrolled effector $CD4^+$ and $CD8^+$ T cell

responses, proving that c-Myc is also necessary for Treg [124]. Surprisingly, c-Myc deficiency affects only the activation of the Tregs, but they present normal survival [102].

2.5 T Cell Metabolism During Exhaustion

Persistent stimulation conditions, like chronic viral infections or the tumor microenvironment, cause T cell exhaustion. These conditions induce the expression of inhibitory molecules which are programmed death (PD)-1, LAG-3, CTLA-4, and TIM-3 in T cells [74, 123, 127]. Exhausted T cells are not able to produce cytokines and to initiate an inflammatory response. The metabolism of exhausted T cells presents a significant suppression of glycolysis and OXPHOS and higher FAO. In addition, exhausted T cells show decreased glucose levels due to Glut1 downregulation and alterations in mitochondrial biogenesis [2, 104]. Inhibition of OXPHOS in activated T cells is linked to T cell exhaustion. Control of mitochondrial oxidative stress during chronic T cell stimulation permitted sustained T cell proliferation and induced genes associated with stem-like progenitor T cells [119].

It has been described that PD-1 could be a metabolic regulator of exhausted T cells. PD-1 levels have been associated with some important transcription factors like T-bet, a repressor of PD-1 [55]; and FoxO1, a positive regulator of PD-1. During chronic infections, T-bet expression is downregulated and FoxO1 expression is upregulated, triggering an increase in PD-1 expression [110]. Importantly, it has been described that PD-1 from exhausted T cells interacts with PD-L1 and PD-L2, inhibiting RAS and PI3K signaling and thus protecting $CD8^+$ T cells from overstimulation by antigen [73, 130]. The blockade of PI3K signaling reduces mTOR signaling, decreasing the glycolytic capacity [1, 107] and up-regulating FAO and lipolysis [87], important characteristics of exhausted cells metabolism.

Beside the lower oxygen consumption rate, exhausted T cells display increased mitochondrial mass [5, 106], ROS production [74] and ultrastructural changes such as elongated mitochondria cristae [5].

3 Cellular Metabolism in B Cells

In the previous years, B cell metabolism has emerged as a very relevant process for the maturation and function of B cells, showing that different B cells subsets present distinct rate of proliferation and longevity and thus they have huge different metabolic requirements [36, 63, 100, 115].

3.1 B Cell Metabolism During Ontogeny

The development of B cells begins from bone marrow hematopoietic precursors that go through different differentiation states until they are mature B cells. During this process, cellular growth and proliferation are regulated by different transcript factors and the IL-7 and pre-BCR signaling pathways [21]. IL-7 signaling activates both mTORC1 and c-Myc, enhancing glycolysis and driving the anabolism necessary for B cell development and proliferation [47, 129]. Consequently, mTORC1-deficient B cell precursors present reduced mitochondrial mass, reduced activation of c-Myc, decreased oxygen consumption and impaired proliferation [129]. Deletion of mTORC1 in early B cell progenitors blocks the large to small pre-B cell transition [47].

Pro-B cells and large pre-B cells are highly proliferative cells and display an elevated energetic demand. They present enhanced glucose uptake, glycolysis and mitochondrial respiration contrasted to other B cells subsets [111, 129]. The EF-hand domain containing protein D1 (EFhd1) is a calcium binding protein located at the inner mitochondrial membrane. EFhd1 is highly expressed in pro-B cells but it is downregulated by the pre-BCR signal. The deletion of EFhd1 causes increased glycolysis, thus the downregulation of EFhd1 by the pre-BCR could be a mechanism to switch on glycolysis in pre-B cells [111].

AMPK is another central regulator of B cell metabolism and has been involved in B cell development. AMPK is activated in response to low nutrients availability and inhibits mTORC1, stopping the cellular growth [14]. AMPK has been associated with the acquisition of quiescence in B cell maturation [33]. Transitional B cells (an intermediate stage for maturation) display higher mitochondrial content, glucose uptake and ROS production, driven by mTORC1 activation. When B cells become fully mature, AMPK is activated and thus mTORC1 is inhibited, dampening the metabolism. Moreover, unlimited mTORC1 activation leads to reduced B cell development and absent of mature B cells in blood in both humans [14, 33, 86] and mice [6, 20]. The loss of the AMPK-mTORC1 signaling blocks the B cell ontogeny in the large pre-B cells stage [86].

3.2 B Cell Metabolism During Activation in GCs

B cells migrate to the germinal centers (GCs) in the spleen to receive presentation from presenter cells and stimuli from T cells. B cell activation leads to clonal expansion and antibody production. B cells activation in the GC requires a precise metabolic control, characterized by an increase in glucose uptake, glycolysis but also mitochondrial mass [18]. OXPHOS is essential for the initial steps of the humoral immune response [121]. Recently, the oxygen availability has been pointed as a key

factor in B cell activation. It has been shown that GCs present a hypoxic microenvironment in which HIF-1α is activated and plays a key role in B cell reprogramming towards glycolysis [48]. Strikingly, sustained hypoxia or HIF-1α stabilization inhibits mTORC1, impairing the proliferation and class-switching of B cells in GCs [18]. Thus, the typical physiology of GCs includes regional hypoxic areas, and HIF-1α-dependent oxygen sensing to regulate critical functions of B cells.

In the GC, B cells receive two signals, CD40 and BCR stimulation, which activate mTORC1 and c-Myc to increase the metabolic activity. mTORC1 is induced in B cells by T cell co-stimulatory signals [32], driving the increase in the glucose uptake and mitochondrial mass, but it is also important for proliferation of activated B cells [32]. c-Myc is induced by both signals in a proportional manner to the amount of T cells signals [27, 34, 69], and plays an critical role in GCs formation and maintenance, driving glycolysis and mitochondrial biogenesis in B cell upon activation [12, 13, 48], however, the importance of c-Myc in the metabolic state of activated B cells requires further investigation. In a similar way than T cells, the switch of B cells towards glycolysis upon antigen presentation is PI3K dependent [28, 32]. Class I PI3K enzymes play key functions in B cell activation. Their role is to phosphorylate lipids from the plasma membrane to generate distinct phosphoinositide products [49]. The protein kinase Cβ (PKC-β) is also required for the induction of glycolysis after BCR stimulation [9]. Accordingly, B cells deficient for PKC-β present decreased medium acidification in cell culture but increased mitochondrial mass and ROS levels upon activation [113]. In addition, the deletion of PKC-β decreases mTORC1 activation, impairing the formation of GC [113]. Glycogen synthase kinase 3 is activated upon the CD40 signal and has an important role on the control of B cell size, mitochondrial biogenesis, glycolysis and production of ROS [48].

The NFkB subunit cREL is a relevant molecule in B cell metabolism. cREL is activated upon BCR or CD40 stimuli and participates in B cell activation and GC maintenance [4, 114]. cREL drives the gene expression of the glycolytic enzymes phosphofructokinase, phosphoglycerate dehydrogenase and other enzymes involved in the oxidation of FA [45].

Importantly, as in T cells, there are anti-inflammatory B cells known as regulatory B (Breg) cells [70, 98]. These cells secrete IL-10 to shut down the inflammatory response and promote T cell differentiation towards Treg. Very recently, it has been shown that metabolism of cholesterol drives the regulatory properties of B cells since the inhibition of the cholesterol synthesis impairs Breg differentiation. This differentiation is controlled by PI3K signaling [8].

3.3 Plasma Cells Differentiation and Antibody Production

Once B cells have proliferated, they produce high amounts of antibodies to fight against pathogens. B cells requires specific metabolic conditions characterized for a proper antibody production. Surprisingly, although glycolysis and glucose uptake are increased in activated B cells, glucose restriction does not affect the antibody

production [121]. Humoral immunity requires huge quantities of amino acids to properly build and fold antibodies. The sugar glycosylation is also very important for antibody production, and the amino acids generates biosynthetic substrates to fuel the necessary endoplasmic reticulum biogenesis [63]. Plasma cells also need lipid synthesis to support the expansion of the endoplasmic reticulum [29] and high glycolytic rate [62] to produce immunoglobulins.

mTORC1 inhibition leads to a reduced expression of the immunoglobulin-binding protein and some important molecules required for protein synthesis, suggesting that mTOR signaling is crucial for plasma cell differentiation and antibody production [52]. The transcription factor Blimp1 has been found as a key molecule in differentiating the plasma cells through the control of cellular metabolism by modulating mTORC1 activity in plasma cells [112]. Strikingly, plasma cells with continuous activation of mTORC1 present an enhanced antibody secretion but shorter lifespan [41]. The let-7 miRNA controls the metabolism and function of plasma cells, by inhibition of glycolysis and antibody production [50]. Let7-deficient LPS stimulated B cells show augmented glucose and glutamine uptake and glycolytic capacity, together with higher antibody production but they do not present changes in OXPHOS.

4 Concluding Remarks

Cellular metabolism is organized by several crucial processes important for biomolecules synthesis and as a source of energy. In adaptive immune cells, metabolism plays an important role controlling their fate and function during their development and differentiation stages. Preservation of a specific metabolic profile is essential for mounting successful adaptive immune response to maintain T and B effector functions and to clear pathogens. Strategies targeting dysfunctional or exhausted T and B cell metabolism could be a promising option for both boosting immune response or dampening autoimmunity.

References

1. Arasanz H, Gato-Cañas M, Zuazo M, Ibañez-Vea M, Breckpot K, Kochan G, Escors D (2017) PD1 signal transduction pathways in T cells. Oncotarget 8(31):51936–51945. https://doi.org/10.18632/oncotarget.17232
2. Balkhi MY (2020) Receptor signaling, transcriptional, and metabolic regulation of T cell exhaustion. Oncoimmunology 9(1):1747349. https://doi.org/10.1080/2162402x.2020.1747349
3. Bantug GR, Fischer M, Grählert J, Balmer ML, Unterstab G, Develioglu L, Steiner R, Zhang L, Costa ASH, Gubser PM, Burgener AV, Sauder U, Löliger J, Belle R, Dimeloe S, Lötscher J, Jauch A, Recher M, Hönger G, Hall MN, Romero P, Frezza C, Hess C (2018) Mitochondria-endoplasmic reticulum contact sites function as immunometabolic hubs that orchestrate the rapid recall response of memory CD8(+) T cells. Immunity 48(3):542-555.e546. https://doi.org/10.1016/j.immuni.2018.02.012

4. Basavarajappa SC, Ramakrishnan P (2020) Regulation of B-cell function by NF-kappaB c-Rel in health and disease. Cell Mol Life Sci. https://doi.org/10.1007/s00018-020-03488-w
5. Bengsch B, Johnson AL, Kurachi M, Odorizzi PM, Pauken KE, Attanasio J, Stelekati E, McLane LM, Paley MA, Delgoffe GM, Wherry EJ (2016) Bioenergetic insufficiencies due to metabolic alterations regulated by the inhibitory receptor PD-1 are an early driver of CD8(+) T cell exhaustion. Immunity 45(2):358–373. https://doi.org/10.1016/j.immuni.2016.07.008
6. Benhamron S, Tirosh B (2011) Direct activation of mTOR in B lymphocytes confers impairment in B-cell maturation andloss of marginal zone B cells. Eur J Immunol 41(8):2390–2396. https://doi.org/10.1002/eji.201041336
7. Berod L, Friedrich C, Nandan A, Freitag J, Hagemann S, Harmrolfs K, Sandouk A, Hesse C, Castro CN, Bähre H, Tschirner SK, Gorinski N, Gohmert M, Mayer CT, Huehn J, Ponimaskin E, Abraham WR, Müller R, Lochner M, Sparwasser T (2014) De novo fatty acid synthesis controls the fate between regulatory T and T helper 17 cells. Nat Med 20(11):1327–1333. https://doi.org/10.1038/nm.3704
8. Bibby JA, Purvis HA, Hayday T, Chandra A, Okkenhaug K, Rosenzweig S, Aksentijevich I, Wood M, Lachmann HJ, Kemper C, Cope AP, Perucha E (2020) Cholesterol metabolism drives regulatory B cell IL-10 through provision of geranylgeranyl pyrophosphate. Nat Commun 11(1):3412. https://doi.org/10.1038/s41467-020-17179-4
9. Blair D, Dufort FJ, Chiles TC (2012) Protein kinase Cβ is critical for the metabolic switch to glycolysis following B-cell antigen receptor engagement. Biochem J 448(1):165–169. https://doi.org/10.1042/bj20121225
10. Böttcher M, Renner K, Berger R, Mentz K, Thomas S, Cardenas-Conejo ZE, Dettmer K, Oefner PJ, Mackensen A, Kreutz M, Mougiakakos D (2018) D-2-hydroxyglutarate interferes with HIF-1α stability skewing T-cell metabolism towards oxidative phosphorylation and impairing Th17 polarization. Oncoimmunology 7(7):e1445454. https://doi.org/10.1080/2162402x.2018.1445454
11. Buck MD, O'Sullivan D, Pearce EL (2015) T cell metabolism drives immunity. J Exp Med 212(9):1345–1360. https://doi.org/10.1084/jem.20151159
12. Calado DP, Sasaki Y, Godinho SA, Pellerin A, Köchert K, Sleckman BP, de Alborán IM, Janz M, Rodig S, Rajewsky K (2012) The cell-cycle regulator c-Myc is essential for the formation and maintenance of germinal centers. Nat Immunol 13(11):1092–1100. https://doi.org/10.1038/ni.2418
13. Caro-Maldonado A, Wang R, Nichols AG, Kuraoka M, Milasta S, Sun LD, Gavin AL, Abel ED, Kelsoe G, Green DR, Rathmell JC (2014) Metabolic reprogramming is required for antibody production that is suppressed in anergic but exaggerated in chronically BAFF-exposed B cells. J Immunol 192(8):3626–3636. https://doi.org/10.4049/jimmunol.1302062
14. Chan LN, Chen Z, Braas D, Lee JW, Xiao G, Geng H, Cosgun KN, Hurtz C, Shojaee S, Cazzaniga V, Schjerven H, Ernst T, Hochhaus A, Kornblau SM, Konopleva M, Pufall MA, Cazzaniga G, Liu GJ, Milne TA, Koeffler HP, Ross TS, Sánchez-García I, Borkhardt A, Yamamoto KR, Dickins RA, Graeber TG, Müschen M (2017) Metabolic gatekeeper function of B-lymphoid transcription factors. Nature 542(7642):479–483. https://doi.org/10.1038/nature21076
15. Chang CH, Curtis JD, Maggi LB Jr, Faubert B, Villarino AV, O'Sullivan D, Huang SC, van der Windt GJ, Blagih J, Qiu J, Weber JD, Pearce EJ, Jones RG, Pearce EL (2013) Posttranscriptional control of T cell effector function by aerobic glycolysis. Cell 153(6):1239–1251. https://doi.org/10.1016/j.cell.2013.05.016
16. Chapman NM, Zeng H, Nguyen TM, Wang Y, Vogel P, Dhungana Y, Liu X, Neale G, Locasale JW, Chi H (2018) mTOR coordinates transcriptional programs and mitochondrial metabolism of activated T(reg) subsets to protect tissue homeostasis. Nat Commun 9(1):2095. https://doi.org/10.1038/s41467-018-04392-5
17. Chen H, Yang T, Zhu L, Zhao Y (2015) Cellular metabolism on T-cell development and function. Int Rev Immunol 34(1):19–33. https://doi.org/10.3109/08830185.2014.902452
18. Cho SH, Raybuck AL, Stengel K, Wei M, Beck TC, Volanakis E, Thomas JW, Hiebert S, Haase VH, Boothby MR (2016) Germinal centre hypoxia and regulation of antibody qualities by a hypoxia response system. Nature 537(7619):234–238. https://doi.org/10.1038/nature19334

19. Chornoguz O, Hagan RS, Haile A, Arwood ML, Gamper CJ, Banerjee A, Powell JD (2017) mTORC1 promotes T-bet phosphorylation to regulate Th1 differentiation. J Immunol 198(10):3939–3948. https://doi.org/10.4049/jimmunol.1601078
20. Ci X, Kuraoka M, Wang H, Carico Z, Hopper K, Shin J, Deng X, Qiu Y, Unniraman S, Kelsoe G, Zhong XP (2015) TSC1 promotes B cell maturation but is dispensable for germinal center formation. PLoS One 10 (5):e0127527. https://doi.org/10.1371/journal.pone.0127527
21. Clark MR, Mandal M, Ochiai K, Singh H (2014) Orchestrating B cell lymphopoiesis through interplay of IL-7 receptor and pre-B cell receptor signalling. Nat Rev Immunol 14(2):69–80. https://doi.org/10.1038/nri3570
22. Dang EV, Barbi J, Yang HY, Jinasena D, Yu H, Zheng Y, Bordman Z, Fu J, Kim Y, Yen HR, Luo W, Zeller K, Shimoda L, Topalian SL, Semenza GL, Dang CV, Pardoll DM, Pan F (2011) Control of T(H)17/T(reg) balance by hypoxia-inducible factor 1. Cell 146(5):772–784. https://doi.org/10.1016/j.cell.2011.07.033
23. de Oliveira Boldrini V, Dos Santos Farias A, Degasperi GR (2019) Deciphering targets of Th17 cells fate: from metabolism to nuclear receptors. Scand J Immunol 90(4):e12793. https://doi.org/10.1111/sji.12793
24. Delgoffe GM, Kole TP, Zheng Y, Zarek PE, Matthews KL, Xiao B, Worley PF, Kozma SC, Powell JD (2009) The mTOR kinase differentially regulates effector and regulatory T cell lineage commitment. Immunity 30(6):832–844. https://doi.org/10.1016/j.immuni.2009.04.014
25. Delgoffe GM, Pollizzi KN, Waickman AT, Heikamp E, Meyers DJ, Horton MR, Xiao B, Worley PF, Powell JD (2011) The kinase mTOR regulates the differentiation of helper T cells through the selective activation of signaling by mTORC1 and mTORC2. Nat Immunol 12(4):295–303. https://doi.org/10.1038/ni.2005
26. Desdín-Micó G, Soto-Heredero G, Aranda JF, Oller J, Carrasco E, Gabandé-Rodríguez E, Blanco EM, Alfranca A, Cussó L, Desco M, Ibañez B, Gortazar AR, Fernández-Marcos P, Navarro MN, Hernaez B, Alcamí A, Baixauli F, Mittelbrunn M (2020) T cells with dysfunctional mitochondria induce multimorbidity and premature senescence. Science 368(6497):1371–1376. https://doi.org/10.1126/science.aax0860
27. Dominguez-Sola D, Victora GD, Ying CY, Phan RT, Saito M, Nussenzweig MC, Dalla-Favera R (2012) The proto-oncogene MYC is required for selection in the germinal center and cyclic reentry. Nat Immunol 13(11):1083–1091. https://doi.org/10.1038/ni.2428
28. Doughty CA, Bleiman BF, Wagner DJ, Dufort FJ, Mataraza JM, Roberts MF, Chiles TC (2006) Antigen receptor-mediated changes in glucose metabolism in B lymphocytes: role of phosphatidylinositol 3-kinase signaling in the glycolytic control of growth. Blood 107(11):4458–4465. https://doi.org/10.1182/blood-2005-12-4788
29. Dufort FJ, Gumina MR, Ta NL, Tao Y, Heyse SA, Scott DA, Richardson AD, Seyfried TN, Chiles TC (2014) Glucose-dependent de novo lipogenesis in B lymphocytes: a requirement for atp-citrate lyase in lipopolysaccharide-induced differentiation. J Biol Chem 289(10):7011–7024. https://doi.org/10.1074/jbc.M114.551051
30. Düvel K, Yecies JL, Menon S, Raman P, Lipovsky AI, Souza AL, Triantafellow E, Ma Q, Gorski R, Cleaver S, Vander Heiden MG, MacKeigan JP, Finan PM, Clish CB, Murphy LO, Manning BD (2010) Activation of a metabolic gene regulatory network downstream of mTOR complex 1. Mol Cell 39(2):171–183. https://doi.org/10.1016/j.molcel.2010.06.022
31. Emambokus N, Granger A, Mott R, Helenius T (2017) The immunometabolism choreography. Cell Metab 26(1):1. https://doi.org/10.1016/j.cmet.2017.06.020
32. Ersching J, Efeyan A, Mesin L, Jacobsen JT, Pasqual G, Grabiner BC, Dominguez-Sola D, Sabatini DM, Victora GD (2017) Germinal center selection and affinity maturation require dynamic regulation of mTORC1 kinase. Immunity 46(6):1045-1058.e1046. https://doi.org/10.1016/j.immuni.2017.06.005
33. Farmer JR, Allard-Chamard H, Sun N, Ahmad M, Bertocchi A, Mahajan VS, Aicher T, Arnold J, Benson MD, Morningstar J, Barmettler S, Yuen G, Murphy SJH, Walter JE, Ghebremichael M, Shalek AK, Batista F, Gerszten R, Pillai S (2019) Induction of metabolic quiescence defines the transitional to follicular B cell switch. Sci Signal 12(604). https://doi.org/10.1126/scisignal.aaw5573

34. Finkin S, Hartweger H, Oliveira TY, Kara EE, Nussenzweig MC (2019) Protein amounts of the MYC transcription factor determine germinal center B cell division capacity. Immunity 51(2):324-336.e325. https://doi.org/10.1016/j.immuni.2019.06.013
35. Finlay DK, Rosenzweig E, Sinclair LV, Feijoo-Carnero C, Hukelmann JL, Rolf J, Panteleyev AA, Okkenhaug K, Cantrell DA (2012) PDK1 regulation of mTOR and hypoxia-inducible factor 1 integrate metabolism and migration of CD8+ T cells. J Exp Med 209(13):2441–2453. https://doi.org/10.1084/jem.20112607
36. Franchina DG, Grusdat M, Brenner D (2018) B-cell metabolic remodeling and cancer. Trends Cancer 4(2):138–150. https://doi.org/10.1016/j.trecan.2017.12.006
37. Fu Z, Ye J, Dean JW, Bostick JW, Weinberg SE, Xiong L, Oliff KN, Chen ZE, Avram D, Chandel NS, Zhou L (2019) Requirement of mitochondrial transcription factor A in tissue-resident regulatory T cell maintenance and function. Cell Rep 28(1):159-171.e154. https://doi.org/10.1016/j.celrep.2019.06.024
38. Geltink RIK, Kyle RL, Pearce EL (2018) Unraveling the complex interplay between T cell metabolism and function. Annu Rev Immunol 36:461–488. https://doi.org/10.1146/annurev-immunol-042617-053019
39. Gerriets VA, Kishton RJ, Nichols AG, Macintyre AN, Inoue M, Ilkayeva O, Winter PS, Liu X, Priyadharshini B, Slawinska ME, Haeberli L, Huck C, Turka LA, Wood KC, Hale LP, Smith PA, Schneider MA, MacIver NJ, Locasale JW, Newgard CB, Shinohara ML, Rathmell JC (2015) Metabolic programming and PDHK1 control CD4+ T cell subsets and inflammation. J Clin Invest 125(1):194–207. https://doi.org/10.1172/JCI76012
40. Gerriets VA, Rathmell JC (2012) Metabolic pathways in T cell fate and function. Trends Immunol 33(4):168–173. https://doi.org/10.1016/j.it.2012.01.010
41. Goldfinger M, Shmuel M, Benhamron S, Tirosh B (2011) Protein synthesis in plasma cells is regulated by crosstalk between endoplasmic reticulum stress and mTOR signaling. Eur J Immunol 41(2):491–502. https://doi.org/10.1002/eji.201040677
42. Gubser PM, Bantug GR, Razik L, Fischer M, Dimeloe S, Hoenger G, Durovic B, Jauch A, Hess C (2013) Rapid effector function of memory CD8+ T cells requires an immediate-early glycolytic switch. Nat Immunol 14(10):1064–1072. https://doi.org/10.1038/ni.2687
43. Hayashi K, Jutabha P, Endou H, Sagara H, Anzai N (2013) LAT1 is a critical transporter of essential amino acids for immune reactions in activated human T cells. J Immunol 191(8):4080–4085. https://doi.org/10.4049/jimmunol.1300923
44. Heinzel S, Marchingo JM, Horton MB, Hodgkin PD (2018) The regulation of lymphocyte activation and proliferation. Curr Opin Immunol 51:32–38. https://doi.org/10.1016/j.coi.2018.01.002
45. Heise N, De Silva NS, Silva K, Carette A, Simonetti G, Pasparakis M, Klein U (2014) Germinal center B cell maintenance and differentiation are controlled by distinct NF-κB transcription factor subunits. J Exp Med 211(10):2103–2118. https://doi.org/10.1084/jem.20132613
46. Howie D, Ten Bokum A, Necula AS, Cobbold SP, Waldmann H (2017) The role of lipid metabolism in T lymphocyte differentiation and survival. Front Immunol 8:1949. https://doi.org/10.3389/fimmu.2017.01949
47. Iwata TN, Ramírez JA, Tsang M, Park H, Margineantu DH, Hockenbery DM, Iritani BM (2016) Conditional disruption of raptor reveals an essential role for mTORC1 in B cell development, survival, and metabolism. J Immunol 197(6):2250–2260. https://doi.org/10.4049/jimmunol.1600492
48. Jellusova J, Cato MH, Apgar JR, Ramezani-Rad P, Leung CR, Chen C, Richardson AD, Conner EM, Benschop RJ, Woodgett JR, Rickert RC (2017) Gsk3 is a metabolic checkpoint regulator in B cells. Nat Immunol 18(3):303–312. https://doi.org/10.1038/ni.3664
49. Jellusova J, Rickert RC (2016) The PI3K pathway in B cell metabolism. Crit Rev Biochem Mol Biol 51(5):359–378. https://doi.org/10.1080/10409238.2016.1215288
50. Jiang S, Yan W, Wang SE, Baltimore D (2018) Let-7 suppresses b cell activation through restricting the availability of necessary nutrients. Cell Metab 27(2):393-403.e394. https://doi.org/10.1016/j.cmet.2017.12.007

51. Johnson MO, Wolf MM, Madden MZ, Andrejeva G, Sugiura A, Contreras DC, Maseda D, Liberti MV, Paz K, Kishton RJ, Johnson ME, de Cubas AA, Wu P, Li G, Zhang Y, Newcomb DC, Wells AD, Restifo NP, Rathmell WK, Locasale JW, Davila ML, Blazar BR, Rathmell JC (2018) Distinct regulation of Th17 and Th1 cell differentiation by glutaminase-dependent metabolism. Cell 175(7):1780–1795 e1719. https://doi.org/10.1016/j.cell.2018.10.001
52. Jones DD, Gaudette BT, Wilmore JR, Chernova I, Bortnick A, Weiss BM, Allman D (2016) mTOR has distinct functions in generating versus sustaining humoral immunity. J Clin Invest 126(11):4250–4261. https://doi.org/10.1172/jci86504
53. Jones RG, Pearce EJ (2017) MenTORing immunity: mTOR signaling in the development and function of tissue-resident immune cells. Immunity 46(5):730–742. https://doi.org/10.1016/j.immuni.2017.04.028
54. Jung J, Zeng H, Horng T (2019) Metabolism as a guiding force for immunity. Nat Cell Biol 21(1):85–93. https://doi.org/10.1038/s41556-018-0217-x
55. Kao C, Oestreich KJ, Paley MA, Crawford A, Angelosanto JM, Ali MA, Intlekofer AM, Boss JM, Reiner SL, Weinmann AS, Wherry EJ (2011) Transcription factor T-bet represses expression of the inhibitory receptor PD-1 and sustains virus-specific CD8+ T cell responses during chronic infection. Nat Immunol 12(7):663–671. https://doi.org/10.1038/ni.2046
56. Kedia-Mehta N, Finlay DK (2019) Competition for nutrients and its role in controlling immune responses. Nat Commun 10(1):2123. https://doi.org/10.1038/s41467-019-10015-4
57. Kidani Y, Bensinger SJ (2017) Reviewing the impact of lipid synthetic flux on Th17 function. Curr Opin Immunol 46:121–126. https://doi.org/10.1016/j.coi.2017.03.012
58. Kishore M, Cheung KCP, Fu H, Bonacina F, Wang G, Coe D, Ward EJ, Colamatteo A, Jangani M, Baragetti A, Matarese G, Smith DM, Haas R, Mauro C, Wraith DC, Okkenhaug K, Catapano AL, De Rosa V, Norata GD, Marelli-Berg FM (2017) Regulatory T cell migration is dependent on glucokinase-mediated glycolysis. Immunity 47(5):875-889.e810. https://doi.org/10.1016/j.immuni.2017.10.017
59. Kishore M, Cheung KCP, Fu H, Bonacina F, Wang G, Coe D, Ward EJ, Colamatteo A, Jangani M, Baragetti A, Matarese G, Smith DM, Haas R, Mauro C, Wraith DC, Okkenhaug K, Catapano AL, De Rosa V, Norata GD, Marelli-Berg FM (2018) Regulatory T cell migration is dependent on glucokinase-mediated glycolysis. Immunity 48(4):831–832. https://doi.org/10.1016/j.immuni.2018.03.034
60. Klysz D, Tai X, Robert PA, Craveiro M, Cretenet G, Oburoglu L, Mongellaz C, Floess S, Fritz V, Matias MI, Yong C, Surh N, Marie JC, Huehn J, Zimmermann V, Kinet S, Dardalhon V, Taylor N (2015) Glutamine-dependent α-ketoglutarate production regulates the balance between T helper 1 cell and regulatory T cell generation. Sci Signal 8(396):ra97. https://doi.org/10.1126/scisignal.aab2610
61. Kurebayashi Y, Nagai S, Ikejiri A, Ohtani M, Ichiyama K, Baba Y, Yamada T, Egami S, Hoshii T, Hirao A, Matsuda S, Koyasu S (2012) PI3K-Akt-mTORC1-S6K1/2 axis controls Th17 differentiation by regulating Gfi1 expression and nuclear translocation of RORgamma. Cell Rep 1(4):360–373. https://doi.org/10.1016/j.celrep.2012.02.007
62. Lam WY, Becker AM, Kennerly KM, Wong R, Curtis JD, Llufrio EM, McCommis KS, Fahrmann J, Pizzato HA, Nunley RM, Lee J, Wolfgang MJ, Patti GJ, Finck BN, Pearce EL, Bhattacharya D (2016) Mitochondrial pyruvate import promotes long-term survival of antibody-secreting plasma cells. Immunity 45(1):60–73. https://doi.org/10.1016/j.immuni.2016.06.011
63. Lam WY, Bhattacharya D (2018) Metabolic links between plasma cell survival, secretion, and stress. Trends Immunol 39(1):19–27. https://doi.org/10.1016/j.it.2017.08.007
64. Lee J, Walsh MC, Hoehn KL, James DE, Wherry EJ, Choi Y (2014) Regulator of fatty acid metabolism, acetyl coenzyme a carboxylase 1, controls T cell immunity. J Immunol 192(7):3190–3199. https://doi.org/10.4049/jimmunol.1302985
65. Lee K, Gudapati P, Dragovic S, Spencer C, Joyce S, Killeen N, Magnuson MA, Boothby M (2010) Mammalian target of rapamycin protein complex 2 regulates differentiation of Th1 and Th2 cell subsets via distinct signaling pathways. Immunity 32(6):743–753. https://doi.org/10.1016/j.immuni.2010.06.002

66. Levine AG, Arvey A, Jin W, Rudensky AY (2014) Continuous requirement for the TCR in regulatory T cell function. Nat Immunol 15(11):1070–1078. https://doi.org/10.1038/ni.3004
67. Lochner M, Berod L, Sparwasser T (2015) Fatty acid metabolism in the regulation of T cell function. Trends Immunol 36(2):81–91. https://doi.org/10.1016/j.it.2014.12.005
68. Loftus RM, Finlay DK (2016) Immunometabolism: cellular metabolism turns immune regulator. J Biol Chem 291(1):1–10. https://doi.org/10.1074/jbc.R115.693903
69. Luo W, Weisel F, Shlomchik MJ (2018) B cell receptor and CD40 signaling are rewired for synergistic induction of the c-Myc transcription factor in germinal center B cells. Immunity 48(2):313-326.e315. https://doi.org/10.1016/j.immuni.2018.01.008
70. Lykken JM, Candando KM, Tedder TF (2015) Regulatory B10 cell development and function. Int Immunol 27(10):471–477. https://doi.org/10.1093/intimm/dxv046
71. Ma EH, Bantug G, Griss T, Condotta S, Johnson RM, Samborska B, Mainolfi N, Suri V, Guak H, Balmer ML, Verway MJ, Raissi TC, Tsui H, Boukhaled G, Henriques da Costa S, Frezza C, Krawczyk CM, Friedman A, Manfredi M, Richer MJ, Hess C, Jones RG (2017) Serine is an essential metabolite for effector T cell expansion. Cell Metab 25(2):345–357. https://doi.org/10.1016/j.cmet.2016.12.011
72. Macintyre AN, Gerriets VA, Nichols AG, Michalek RD, Rudolph MC, Deoliveira D, Anderson SM, Abel ED, Chen BJ, Hale LP, Rathmell JC (2014) The glucose transporter Glut1 is selectively essential for CD4 T cell activation and effector function. Cell Metab 20(1):61–72. https://doi.org/10.1016/j.cmet.2014.05.004
73. Marasco M, Berteotti A, Weyershaeuser J, Thorausch N, Sikorska J, Krausze J, Brandt HJ, Kirkpatrick J, Rios P, Schamel WW, Köhn M, Carlomagno T (2020) Molecular mechanism of SHP2 activation by PD-1 stimulation. Sci Adv 6(5):eaay4458. https://doi.org/10.1126/sciadv.aay4458
74. McKinney EF, Smith KGC (2018) Metabolic exhaustion in infection, cancer and autoimmunity. Nat Immunol 19(3):213–221. https://doi.org/10.1038/s41590-018-0045-y
75. Menk AV, Scharping NE, Moreci RS, Zeng X, Guy C, Salvatore S, Bae H, Xie J, Young HA, Wendell SG, Delgoffe GM (2018) Early TCR signaling induces rapid aerobic glycolysis enabling distinct acute T cell effector functions. Cell Rep 22(6):1509–1521. https://doi.org/10.1016/j.celrep.2018.01.040
76. Michalek RD, Gerriets VA, Jacobs SR, Macintyre AN, MacIver NJ, Mason EF, Sullivan SA, Nichols AG, Rathmell JC (2011) Cutting edge: distinct glycolytic and lipid oxidative metabolic programs are essential for effector and regulatory CD4+ T cell subsets. J Immunol 186(6):3299–3303. https://doi.org/10.4049/jimmunol.1003613
77. Miska J, Lee-Chang C, Rashidi A, Muroski ME, Chang AL, Lopez-Rosas A, Zhang P, Panek WK, Cordero A, Han Y, Ahmed AU, Chandel NS, Lesniak MS (2019) HIF-1α is a metabolic switch between glycolytic-driven migration and oxidative phosphorylation-driven immunosuppression of tregs in glioblastoma. Cell Rep 27(1):226-237.e224. https://doi.org/10.1016/j.celrep.2019.03.029
78. Munford H, Dimeloe S (2019) Intrinsic and extrinsic determinants of T cell metabolism in health and disease. Front Mol Biosci 6:118. https://doi.org/10.3389/fmolb.2019.00118
79. Nakaya M, Xiao Y, Zhou X, Chang JH, Chang M, Cheng X, Blonska M, Lin X, Sun SC (2014) Inflammatory T cell responses rely on amino acid transporter ASCT2 facilitation of glutamine uptake and mTORC1 kinase activation. Immunity 40(5):692–705. https://doi.org/10.1016/j.immuni.2014.04.007
80. Newton R, Priyadharshini B, Turka LA (2016) Immunometabolism of regulatory T cells. Nat Immunol 17(6):618–625. https://doi.org/10.1038/ni.3466
81. Nicoli F, Papagno L, Frere JJ, Cabral-Piccin MP, Clave E, Gostick E, Toubert A, Price DA, Caputo A, Appay V (2018) Naïve CD8(+) T-cells engage a versatile metabolic program upon activation in humans and differ energetically from memory CD8(+) T-cells. Front Immunol 9:2736. https://doi.org/10.3389/fimmu.2018.02736
82. Norata GD, Caligiuri G, Chavakis T, Matarese G, Netea MG, Nicoletti A, O'Neill LA, Marelli-Berg FM (2015) The cellular and molecular basis of translational immunometabolism. Immunity 43(3):421–434. https://doi.org/10.1016/j.immuni.2015.08.023

83. O'Neill L (2017) Immunometabolism and the land of milk and honey. Nat Rev Immunol 17(4):217. https://doi.org/10.1038/nri.2017.22
84. O'Sullivan D, van der Windt GJ, Huang SC, Curtis JD, Chang CH, Buck MD, Qiu J, Smith AM, Lam WY, DiPlato LM, Hsu FF, Birnbaum MJ, Pearce EJ, Pearce EL (2014) Memory CD8(+) T cells use cell-intrinsic lipolysis to support the metabolic programming necessary for development. Immunity 41(1):75–88. https://doi.org/10.1016/j.immuni.2014.06.005
85. Oestreich KJ, Read KA, Gilbertson SE, Hough KP, McDonald PW, Krishnamoorthy V, Weinmann AS (2014) Bcl-6 directly represses the gene program of the glycolysis pathway. Nat Immunol 15(10):957–964. https://doi.org/10.1038/ni.2985
86. Park H, Staehling K, Tsang M, Appleby MW, Brunkow ME, Margineantu D, Hockenbery DM, Habib T, Liggitt HD, Carlson G, Iritani BM (2012) Disruption of Fnip1 reveals a metabolic checkpoint controlling B lymphocyte development. Immunity 36(5):769–781. https://doi.org/10.1016/j.immuni.2012.02.019
87. Patsoukis N, Bardhan K, Chatterjee P, Sari D, Liu B, Bell LN, Karoly ED, Freeman GJ, Petkova V, Seth P, Li L, Boussiotis VA (2015) PD-1 alters T-cell metabolic reprogramming by inhibiting glycolysis and promoting lipolysis and fatty acid oxidation. Nat Commun 6:6692. https://doi.org/10.1038/ncomms7692
88. Pearce EJ, Pearce EL (2018) Immunometabolism in 2017: driving immunity: all roads lead to metabolism. Nat Rev Immunol 18(2):81–82. https://doi.org/10.1038/nri.2017.139
89. Pearce EL, Pearce EJ (2013) Metabolic pathways in immune cell activation and quiescence. Immunity 38(4):633–643. https://doi.org/10.1016/j.immuni.2013.04.005
90. Pearce EL, Walsh MC, Cejas PJ, Harms GM, Shen H, Wang LS, Jones RG, Choi Y (2009) Enhancing CD8 T-cell memory by modulating fatty acid metabolism. Nature 460(7251):103–107. https://doi.org/10.1038/nature08097
91. Pollizzi KN, Patel CH, Sun IH, Oh MH, Waickman AT, Wen J, Delgoffe GM, Powell JD (2015) mTORC1 and mTORC2 selectively regulate $CD8^+$ T cell differentiation. J Clin Invest 125(5):2090–2108. https://doi.org/10.1172/jci77746
92. Powell JD, Pollizzi KN, Heikamp EB, Horton MR (2012) Regulation of immune responses by mTOR. Annu Rev Immunol 30:39–68. https://doi.org/10.1146/annurev-immunol-020711-075024
93. Procaccini C, De Rosa V, Galgani M, Abanni L, Calì G, Porcellini A, Carbone F, Fontana S, Horvath TL, La Cava A, Matarese G (2010) An oscillatory switch in mTOR kinase activity sets regulatory T cell responsiveness. Immunity 33(6):929–941. https://doi.org/10.1016/j.immuni.2010.11.024
94. Puleston DJ, Villa M, Pearce EL (2017) Ancillary activity: beyond core metabolism in immune cells. Cell Metab 26(1):131–141. https://doi.org/10.1016/j.cmet.2017.06.019
95. Ramalho R, Rao M, Zhang C, Agrati C, Ippolito G, Wang FS, Zumla A, Maeurer M (2020) Immunometabolism: new insights and lessons from antigen-directed cellular immune responses. Semin Immunopathol 42(3):279–313. https://doi.org/10.1007/s00281-020-00798-w
96. Rolf J, Zarrouk M, Finlay DK, Foretz M, Viollet B, Cantrell DA (2013) AMPKα1: a glucose sensor that controls CD8 T-cell memory. Eur J Immunol 43(4):889–896. https://doi.org/10.1002/eji.201243008
97. Ron-Harel N, Ghergurovich JM, Notarangelo G, LaFleur MW, Tsubosaka Y, Sharpe AH, Rabinowitz JD, Haigis MC (2019) T cell activation depends on extracellular alanine. Cell Rep 28(12):3011–3021 e3014. https://doi.org/10.1016/j.celrep.2019.08.034
98. Rosser EC, Mauri C (2015) Regulatory B cells: origin, phenotype, and function. Immunity 42(4):607–612. https://doi.org/10.1016/j.immuni.2015.04.005
99. Rothenberg EV (2014) Transcriptional control of early T and B cell developmental choices. Annu Rev Immunol 32:283–321. https://doi.org/10.1146/annurev-immunol-032712-100024
100. Sacks D, Baxter B, Campbell BCV, Carpenter JS, Cognard C, Dippel D, Eesa M, Fischer U, Hausegger K, Hirsch JA, Shazam Hussain M, Jansen O, Jayaraman MV, Khalessi AA, Kluck BW, Lavine S, Meyers PM, Ramee S, Rüfenacht DA, Schirmer CM, Vorwerk D (2018) Multisociety consensus quality improvement revised consensus statement for endovascular

therapy of acute ischemic stroke. Int J Stroke 13(6):612–632. https://doi.org/10.1177/1747493018778713
101. Salmond RJ (2018) mTOR regulation of glycolytic metabolism in T cells. Front Cell Dev Biol 6:122. https://doi.org/10.3389/fcell.2018.00122
102. Saravia J, Zeng H, Dhungana Y, Bastardo Blanco D, Nguyen TM, Chapman NM, Wang Y, Kanneganti A, Liu S, Raynor JL, Vogel P, Neale G, Carmeliet P, Chi H (2020) Homeostasis and transitional activation of regulatory T cells require c-Myc. Sci Adv 6(1):eaaw6443. https://doi.org/10.1126/sciadv.aaw6443
103. Saxton RA, Sabatini DM (2017) mTOR signaling in growth, metabolism, and disease. Cell 168(6):960–976. https://doi.org/10.1016/j.cell.2017.02.004
104. Scharping NE, Menk AV, Moreci RS, Whetstone RD, Dadey RE, Watkins SC, Ferris RL, Delgoffe GM (2016) The tumor microenvironment represses T cell mitochondrial biogenesis to drive intratumoral T cell metabolic insufficiency and dysfunction. Immunity 45(2):374–388. https://doi.org/10.1016/j.immuni.2016.07.009
105. Schett G, Neurath MF (2018) Resolution of chronic inflammatory disease: universal and tissue-specific concepts. Nat Commun 9(1):3261. https://doi.org/10.1038/s41467-018-05800-6
106. Schurich A, Pallett LJ, Jajbhay D, Wijngaarden J, Otano I, Gill US, Hansi N, Kennedy PT, Nastouli E, Gilson R, Frezza C, Henson SM, Maini MK (2016) Distinct metabolic requirements of exhausted and functional virus-specific CD8 T cells in the same host. Cell Rep 16(5):1243–1252. https://doi.org/10.1016/j.celrep.2016.06.078
107. Sears JD, Waldron KJ, Wei J, Chang CH (2020) Targeting metabolism to reverse T-cell exhaustion in chronic viral infections. Immunology. https://doi.org/10.1111/imm.13238
108. Sena LA, Li S, Jairaman A, Prakriya M, Ezponda T, Hildeman DA, Wang CR, Schumacker PT, Licht JD, Perlman H, Bryce PJ, Chandel NS (2013) Mitochondria are required for antigen-specific T cell activation through reactive oxygen species signaling. Immunity 38(2):225–236. https://doi.org/10.1016/j.immuni.2012.10.020
109. Shi LZ, Wang R, Huang G, Vogel P, Neale G, Green DR, Chi H (2011) HIF1alpha-dependent glycolytic pathway orchestrates a metabolic checkpoint for the differentiation of TH17 and Treg cells. J Exp Med 208(7):1367–1376. https://doi.org/10.1084/jem.20110278
110. Staron MM, Gray SM, Marshall HD, Parish IA, Chen JH, Perry CJ, Cui G, Li MO, Kaech SM (2014) The transcription factor FoxO1 sustains expression of the inhibitory receptor PD-1 and survival of antiviral CD8(+) T cells during chronic infection. Immunity 41(5):802–814. https://doi.org/10.1016/j.immuni.2014.10.013
111. Stein M, Dütting S, Mougiakakos D, Bösl M, Fritsch K, Reimer D, Urbanczyk S, Steinmetz T, Schuh W, Bozec A, Winkler TH, Jäck HM, Mielenz D (2017) A defined metabolic state in pre B cells governs B-cell development and is counterbalanced by Swiprosin-2/EFhd1. Cell Death Differ 24(7):1239–1252. https://doi.org/10.1038/cdd.2017.52
112. Tellier J, Shi W, Minnich M, Liao Y, Crawford S, Smyth GK, Kallies A, Busslinger M, Nutt SL (2016) Blimp-1 controls plasma cell function through the regulation of immunoglobulin secretion and the unfolded protein response. Nat Immunol 17(3):323–330. https://doi.org/10.1038/ni.3348
113. Tsui C, Martinez-Martin N, Gaya M, Maldonado P, Llorian M, Legrave NM, Rossi M, MacRae JI, Cameron AJ, Parker PJ, Leitges M, Bruckbauer A, Batista FD (2018) Protein kinase C-β dictates B cell fate by regulating mitochondrial remodeling, metabolic reprogramming, and heme biosynthesis. Immunity 48(6):1144-1159.e1145. https://doi.org/10.1016/j.immuni.2018.04.031
114. Tumang JR, Owyang A, Andjelic S, Jin Z, Hardy RR, Liou ML, Liou HC (1998) c-Rel is essential for B lymphocyte survival and cell cycle progression. Eur J Immunol 28(12):4299–4312. https://doi.org/10.1002/(sici)1521-4141(199812)28:12%3c4299::Aid-immu4299%3e3.0.Co;2-y
115. Urbanczyk S, Stein M, Schuh W, Jäck HM, Mougiakakos D, Mielenz D (2018) Regulation of Energy Metabolism during Early B Lymphocyte Development. Int J Mol Sci 19(8). https://doi.org/10.3390/ijms19082192

116. van der Windt GJ, Everts B, Chang CH, Curtis JD, Freitas TC, Amiel E, Pearce EJ, Pearce EL (2012) Mitochondrial respiratory capacity is a critical regulator of CD8+ T cell memory development. Immunity 36(1):68–78. https://doi.org/10.1016/j.immuni.2011.12.007
117. van der Windt GJ, O'Sullivan D, Everts B, Huang SC, Buck MD, Curtis JD, Chang CH, Smith AM, Ai T, Faubert B, Jones RG, Pearce EJ, Pearce EL (2013) CD8 memory T cells have a bioenergetic advantage that underlies their rapid recall ability. Proc Natl Acad Sci USA 110(35):14336–14341. https://doi.org/10.1073/pnas.1221740110
118. van der Windt GJ, Pearce EL (2012) Metabolic switching and fuel choice during T-cell differentiation and memory development. Immunol Rev 249(1):27–42. https://doi.org/10.1111/j.1600-065X.2012.01150.x
119. Vardhana SA, Hwee MA, Berisa M, Wells DK, Yost KE, King B, Smith M, Herrera PS, Chang HY, Satpathy AT, van den Brink MRM, Cross JR, Thompson CB (2020) Impaired mitochondrial oxidative phosphorylation limits the self-renewal of T cells exposed to persistent antigen. Nat Immunol. https://doi.org/10.1038/s41590-020-0725-2
120. Wang R, Dillon CP, Shi LZ, Milasta S, Carter R, Finkelstein D, McCormick LL, Fitzgerald P, Chi H, Munger J, Green DR (2011) The transcription factor Myc controls metabolic reprogramming upon T lymphocyte activation. Immunity 35(6):871–882. https://doi.org/10.1016/j.immuni.2011.09.021
121. Waters LR, Ahsan FM, Wolf DM, Shirihai O, Teitell MA (2018) Initial B cell activation induces metabolic reprogramming and mitochondrial remodeling. iScience 5:99–109. https://doi.org/10.1016/j.isci.2018.07.005
122. Weinberg SE, Singer BD, Steinert EM, Martinez CA, Mehta MM, Martínez-Reyes I, Gao P, Helmin KA, Abdala-Valencia H, Sena LA, Schumacker PT, Turka LA, Chandel NS (2019) Mitochondrial complex III is essential for suppressive function of regulatory T cells. Nature 565(7740):495–499. https://doi.org/10.1038/s41586-018-0846-z
123. Wherry EJ (2011) T cell exhaustion. Nat Immunol 12(6):492–499. https://doi.org/10.1038/ni.2035
124. Wu Y, Xia R, Dai C, Yan S, Xie T, Liu B, Gan L, Zhuang Z, Huang Q (2019) Dexamethasone inhibits the proliferation of tumor cells. Cancer Manage Res 11:1141–1154. https://doi.org/10.2147/cmar.S187659
125. Yang K, Blanco DB, Chen X, Dash P, Neale G, Rosencrance C, Easton J, Chen W, Cheng C, Dhungana Y, Kc A, Awad W, Guo XJ, Thomas PG, Chi H (2018) Metabolic signaling directs the reciprocal lineage decisions of alphabeta and gammadelta T cells. Sci Immunol 3(25). https://doi.org/10.1126/sciimmunol.aas9818
126. Yang K, Shrestha S, Zeng H, Karmaus PW, Neale G, Vogel P, Guertin DA, Lamb RF, Chi H (2013) T cell exit from quiescence and differentiation into Th2 cells depend on Raptor-mTORC1-mediated metabolic reprogramming. Immunity 39(6):1043–1056. https://doi.org/10.1016/j.immuni.2013.09.015
127. Ye B, Liu X, Li X, Kong H, Tian L, Chen Y (2015) T-cell exhaustion in chronic hepatitis B infection: current knowledge and clinical significance. Cell Death Dis 6(3):e1694. https://doi.org/10.1038/cddis.2015.42
128. Zeng H, Yang K, Cloer C, Neale G, Vogel P, Chi H (2013) mTORC1 couples immune signals and metabolic programming to establish T(reg)-cell function. Nature 499(7459):485–490. https://doi.org/10.1038/nature12297
129. Zeng H, Yu M, Tan H, Li Y, Su W, Shi H, Dhungana Y, Guy C, Neale G, Cloer C, Peng J, Wang D, Chi H (2018) Discrete roles and bifurcation of PTEN signaling and mTORC1-mediated anabolic metabolism underlie IL-7-driven B lymphopoiesis. Sci Adv 4(1):eaar5701. https://doi.org/10.1126/sciadv.aar5701
130. Zhao M, Guo W, Wu Y, Yang C, Zhong L, Deng G, Zhu Y, Liu W, Gu Y, Lu Y, Kong L, Meng X, Xu Q, Sun Y (2019) SHP2 inhibition triggers anti-tumor immunity and synergizes with PD-1 blockade. Acta Pharm Sin B 9(2):304–315. https://doi.org/10.1016/j.apsb.2018.08.009

Obesity, Metabolic Syndrome and Inflammation

Jennifer Lee

1 Introduction

Initially described in the 1960s and 1970s, Metabolic Syndrome represents a clustering of medical conditions, including increased waist circumference and elevated fasting glycemia and triglycerides, which are used to predict increased risk for cardiovascular disease-associated disorders (American Heart Association, 2019). Obesity, one of the metabolic disorders that is clustered with Metabolic Syndrome, is a global health and financial burden affecting nearly 40% of adults aged 18 year and over, and 1 in 5 adolescents aged 5–19 years of age (World Health Organization). The etiology and pathophysiology underlying obesity and insulin resistance are multifactorial and complex. Genome-wide association studies (GWAS) have identified candidate genes and genetic variants that strongly associate with and may contribute to metabolic disease [3, 4, 65, 67, 75]. More recent studies using Mendelian Randomization analyses demonstrate causality of candidate genes with metabolic disease in humans [35, 42]. Preclinical studies using diet-induced and genetically obese rodent models have identified pro-inflammatory molecular culprits and their associated mechanisms that dysregulate glucose homeostasis in different metabolic tissues. Consistently, these pro-inflammatory biomarkers are elevated in humans [18, 29, 72, 83, 88]. In this review, we discuss the effects of key molecular players that contribute to Metabolic Syndrome. Despite efforts to target pro-inflammatory cells or associated molecules that initiate metabolic disease pathogenesis, the prevalence of obesity-associated metabolic disease remains a global epidemic and current therapeutic modalities are not sufficient to improve and/or sustain metabolic goals.

Classically, the immune system plays a critical role in preventing or limiting infection in the host. More recently, is it increasingly recognized that the immune

J. Lee (✉)
Division of Endocrinology, Diabetes and Metabolism, Beth Israel Deconess Medical Center, Harvard Medical School, Boston, MA, USA
e-mail: jlee45@bidmc.harvard.edu

N. O. S. Camara et al. (eds.), *Essential Aspects of Immunometabolism in Health and Disease*, https://doi.org/10.1007/978-3-030-86684-6_7

system contributes to the onset and development of obesity and metabolic disease. Subclinical inflammation is strongly associated with increased risk for cardiovascular events in humans [18, 29, 83, 88]. This section will focus on reviewing known pro-inflammatory molecules and their mechanistic processes that alters the structure and function of white adipose tissue and the gastrointestinal tract. As well, we will examine how these diverse processes intersect and contribute to impaired metabolic homeostasis in states of obesity and insulin resistance. We will also discuss the translational potential of immune-mediated targets for treating metabolic disease and the future direction of immunometabolism research.

2 Adipose Tissue Inflammation and Obesity

White adipose tissue (AT) has been largely redefined as an organ that stores excess energy in the form of lipid to become a highly heterogeneous tissue with important endocrine, thermogenic, and immune functions that contributes to systemic metabolic homeostasis. Adipose tissue synthesizes and secretes a broad repertoire of adipokines, adipocyte-secreted proteins that have pro- and anti-inflammatory properties in response to changes in their local and systemic environment. Many lines of evidence support the roles for multiple adipokines in the pathogenesis of obesity and insulin resistance. Here, we will discuss the role of three key adipokines: tumor necrosis factor-a (TNFa), retinol-binding protein-4 (RBP-4), and leptin, as well as the role of resident macrophages and other immune cell types in adipose tissue, and their contributions to obesity-induced insulin resistance.

3 The Role of Tumor Necrosis Factor (TNF) in Obesity and Insulin Resistance

The identification of adipose tissue-derived TNFa in obesity-associated insulin resistance in 1993 was a major discovery in the field by providing a direct link between pro-inflammatory cytokines and insulin resistance [25]. The fact that TNFa gene expression was elevated in adipose tissue of genetically obese animals, and systemic neutralization of TNFa or genetic deletion of TNFa in mice improved insulin sensitivity demonstrated the importance of this cytokine in regulating insulin sensitivity [25]. Subsequent studies found that adipose tissue expression of TNFa is elevated in obese and insulin-resistant humans [24]. As well, loss of functional TNFa in obese mice increased the activity of the insulin receptor tyrosine kinase in muscle and adipose tissue and increased levels of GLUT4 in muscle [61, 82]. The fact that human adipose tissue makes and secretes TNFa in response to lipopolysaccharide (LPS) ex vivo [68], and that human adipocytes express the toll-like receptor-4

(TLR4) [84], further supports the importance of TNFa in contributing to the low-grade inflammation seen in obesity-induced insulin resistance [70]. The identification of other cytokines, such as IL6, that are also secreted by adipocytes and are critical for regulating innate immunity [5], demonstrates the importance of adipose tissue in contributing to innate immune responses.

Altogether, pro-inflammatory, adipocyte-derived cytokines play a critical role in insulin resistance development. However, translational efforts to target TNFa for the treatment of obesity-induced insulin resistance have been difficult due to broad immune effects of TNFa in different tissue and cell types.

4 The Role of Retinol-Binding Protein-4 (RBP-4) in Insulin Resistance

RBP-4 is a protein secreted predominantly from adipocytes [89] and is a clinical marker of insulin resistance and metabolic syndrome. Serum levels of RBP-4 are elevated in insulin-resistant mice, and this elevation is also observed and highly associates with insulin resistance in humans [13, 21, 89]. Serum RBP-4 is reduced with weight loss in obese people [22], and the reduction of these levels with exercise training is strongly correlated with improved insulin sensitivity [38]. Increased RBP-4 expression in insulin-resistant mice causes white adipose tissue inflammation, and this is mediated at least in part due to RBP-4 mediated activation of CD206+ macrophages and subsequent CD4+ Th1 polarization [55]. The contribution of pro-inflammatory adipose tissue (AT) macrophages to cause systemic insulin resistance is further supported by the fact that adoptive transfer of RBP-4 activated AT antigen presenting cells, including macrophages, into normal mice is sufficient to induce AT inflammation, insulin resistance and glucose intolerance [55]. In insulin-resistant mice, preventing APC activation with administration of cytotoxic T-lymphocyte antigen 4 (CTLA-4), a protein receptor on regulatory T-cells whose expression is upregulated upon T-cell activation, restored glucose intolerance and insulin, demonstrating the major contribution of AT APCs in regulating glucose homeostasis. These studies support the translational potential to target mechanisms that decrease low-grade AT inflammation to thereby restore metabolic homeostasis.

5 The Role of Leptin in Obesity and Insulin Resistance

In 1995, the major discovery of leptin, the ob gene protein product, demonstrated the importance of adipose tissue as an endocrine organ that regulates energy homeostasis [23]. Leptin is an anorectic hormone that is produced primarily from adipocytes [23, 49] and whose circulating levels in mice and humans strongly associate with adiposity and serves a critical role in central regulation of systemic energy balance [19]. In

rodent models of diet-induced obesity and obese humans, circulating leptin levels are elevated yet caloric intake is not reduced with no decrease in body weight gain [19]. Similar to insulin resistance, these studies demonstrated leptin resistance in the hypothalamus and as well as in peripheral tissues. Elevated leptin levels and its associated pro-inflammatory metabolic sequelae have been implicated in the pathophysiology of obesity-related complications, including cardiovascular events and Metabolic Syndrome [7, 37]. Leptin also plays an important role in regulating immunometabolic processes by having direct effects on adaptive immune cells. For example, leptin is necessary for dendritic cell maturation to promote T cell (T helper 17 and T-regulatory) proliferation [54]. States of overnutrition and elevated leptin levels impair adaptive and innate immune responses and this contributes to the low-grade inflammation seen in obesity and insulin resistance.

6 Immune Cell Function Within Adipose Tissue

The immunometabolic capacity and function of immune cells that reside in adipose tissue are dependent on host nutritional status. The proportion of adipose tissue macrophages (ATMs) ranges from 10 to 50% in lean versus diet-induced obese mice and this observation is also consistent in obese humans [87]. In states of nutritional excess and obesity, resident macrophages in adipose tissue are activated by a host of metabolic stimuli [6, 69]. Polarization of macrophages to a pro-inflammatory (M1) or anti-inflammatory (M2) phenotype plays a key role in regulating adipose tissue function [69]. The proof-of-concept experiment that acute endotoxemia is sufficient to cause adipose tissue inflammation and insulin resistance in otherwise healthy adults demonstrates the major contribution of adipose inflammation in metabolic disease pathology [52]. Downstream sequelae of activated T-cells in adipose tissue includes the recruitment of pro-inflammatory M1 macrophages to the target organ, increased levels of pro-inflammatory cytokines, and the development of insulin resistance. As well, the pro-inflammatory bacterial product lipopolysaccharide (LPS) changes macrophage metabolism from oxidative phosphorylation to glycolysis and increases inflammation through a succinate-driven interleukin-1b pathway [76]. To further demonstrate the importance of the innate immune system in regulating systemic glucose homeostasis, deletion of T-cells, associated pro-inflammatory cytokines, or toll-like receptors alters glucose homeostasis in diet-induced obese mice [11]. Identification of distinct transcriptomic signatures of macrophages and other activated immune cells in adipose tissue from normal and obese mice along with functional data demonstrating changes in glycolytic metabolism in these cells supports the idea that the metabolic capacity of AT immune cells in addition to their classical immune functions are important for and affects host metabolism [6]. Furthermore, Th17 cells from diabetic humans have impaired mitochondrial function, altered cytokine production, and generate ATP through glycolysis versus the classical fatty acid oxidation [59], revealing yet another level of immunometabolic complexity underlying insulin resistance and Type 2 Diabetes pathology.

Immune cells play an important role in adipose tissue remodeling in obesity. Adipose tissue inflammation, determined as having elevated levels of ATMs and increased TNFa expression, increases the frequency of adipocyte death in diet-induced obese mice [74]. Crown-like structures, a histological hallmark of adipose tissue inflammation and obesity, are characterized by macrophages that surround apoptotic adipocytes [15, 58]. These ATMs serve to scavenge residual lipids from dying adipocytes and resultantly, excess lipid availability in ATMs in turn alters their cellular metabolism to preferentially increase glycolytic metabolism over the traditional TCA cycle [77]. Depending on the disease pathogenesis, metabolic reprogramming of immune cells promotes change from an anti-inflammatory to pro-inflammatory or altered metabolic state. This major observation has changed the field of immunometabolism and cancer research and has opened up a new multidisciplinary approach to identify therapeutic targets to mitigate disease progression.

Adipose-resident invariant natural killer T-cells (iNKT) are an anti-inflammatory, lipid-sensing immune cell type that plays an important role in regulating inflammatory responses. iNKT cells are reduced in obesity and cell numbers are restored with weight loss [47]. The importance of iNKT cells in regulating metabolic homeostasis is supported by the fact that mice lacking this cell type are heavier with greater adiposity and are insulin resistant, while adoptive transfer of iNKT cells into obese mice is sufficient to improve insulin sensitivity and decrease adiposity and circulating triglyceride levels [47]. Furthermore, activation of iNKT cells is required for improved glucose homeostasis and weight loss in obese mice [45]. The unique transcriptional program of iNKT cells induces an anti-inflammatory phenotype in resident ATMs to downregulate Treg proliferation in adipose tissue [46]. These data demonstrate the importance of iNKT cells in regulating adipose tissue function and metabolic homeostasis.

In summary, this section highlights the role of some key adipokines and immune cell types in adipose tissue in metabolic homeostasis and systemic inflammation. The power of next generation sequencing followed by functional characterization of previously unknown cell types and adipo-markers may lead to the identification of new therapeutic targets with which to treat or prevent metabolic disease [64]. However, attempts to translate adipose-derived molecular and/or resident immune cell targets in adipose tissue have proven difficult. More work investigating the cross-talk between adipose tissue, adipokine-responsive tissues, and the innate immune system in response to adipose-derived biomolecules is needed.

7 The Role of the Gut in Obesity and Inflammation

The gastrointestinal tract is comprised of a heterogeneous population of cells with endocrine, immune and absorptive properties, altogether making the gut an important contributor to glucose homeostasis, systemic inflammation, and host metabolism. The gut microbiome, made up of trillions of functional bacteria and co-existing symbiotically with the host, plays a critical role in maintaining metabolic host health.

The microbiome is a dynamic organ that has evolved with the host to symbiotically utilize ingested nutrients and synthesize functional metabolites with physiologic effects that contribute to regulating host metabolism and homeostasis [28, 36, 63, 80, 81]. In this section, we will focus on how gut enteroendocrine cells, intestinal immune cells, and the gut microbiome respond to different nutritional states and how their function is impaired in obesity-induced insulin resistance. As well, we will highlight key molecular targets in the gut that are currently used for treating Type 2 Diabetes or have translational potential for restoring glucose homeostasis.

8 The Gut is an Immunoendocrine Organ

Enteroendocrine cells (EECs) within the gastrointestinal tract make up the largest endocrine organ in the body. Making up only 1% of the intestinal cell population, these specialized cells are highly dispersed along the length of the gastrointestinal tract and secrete hormones that are critical for regulating food intake and glucose metabolism. In this section, we will focus on gut hormones that play important roles in maintaining intestinal homeostasis, regulating glucose metabolism, and systemic immune responses.

8.1 Proglucagon-Derived Peptides: Glucagon-Like Peptides-1 and -2 (GLP-1 and GLP-2)

Glucagon-like peptide-1 (GLP-1) is secreted from enteroendocrine cells with beneficial incretin effects to augment glucose-stimulated insulin secretion (ref). Beyond the well-established physiological functions of GLP-1 in decreasing food intake, delaying gastric emptying, and inhibiting glucagon secretion, GLP-1 also has anti-inflammatory actions on peripheral metabolic tissues [33]. GLP-1 receptor (GLP1-R) agonists are an effective class of therapeutics for the treatment of Type 2 Diabetes as well as obesity. The associated reductions in systemic inflammation seen with GLP-1R agonist treatment in rodents and humans further highlights the importance of GLP-1 action in metabolic homeostasis. Intraepithelial lymphocytes (IELs) along the gut express GLP-1R and protect the gut from DSS-induced colitis in mice [91]. This is demonstrated by the fact that colonic expression of pro-inflammatory genes in GLP1r-/- mice treated with DSS treatment are reduced following bone marrow transplantation from wild-type mice with functional GLP-1R [91].

GLP-1R is also expressed in other immune cell types in different metabolic tissues. As mentioned previously, in adipose tissue, GLP-1 activates and augments proliferation of resident invariant natural killer T (iNKT) cells, and these beneficial effects increase weight loss in an FGF21-dependent mechanism and decreases adipose tissue inflammation in obese mice [45]. Similarly, ob/ob mice, a genetic model of

diabetes, treated with an adenovirus producing GLP-1 had attenuated adipose tissue macrophage infiltration and inflammation [34].

Moreover, the anti-inflammatory effects of GLP-1 may also be linked to the cardioprotection seen in mice treated with GLP-1R agonists. GLP-1 treatment inhibited aortic monocytic adhesion and attenuated the development of atherosclerosis in wild-type and apolipoprotein E knockout mice [2]. Similarly, GLP-1R agonists suppressed foam cell formation in human monocyte-derived macrophages [78]. Altogether, the extrapancreatic effects of GLP-1 to act on various immune cell types to decrease systemic inflammation demonstrate the broad application of GLP-1-based therapeutics beyond glucose regulation to improve metabolic outcomes.

8.2 Glucagon-Like Peptide-2

Glucagon-like peptide-2 (GLP-2) is also derived from the glucagon gene but unlike the incretin effects of GLP-1, GLP-2 is the most robust intestinotrophic hormone in the gut with nutrient absorptive effects [31, 53]. Mice chronically treated with GLP-2 have increased small intestinal villi height and crypt cell proliferation, demonstrating the direct proliferative effects of GLP-2 on gut growth [32, 51]. Acute GLP-2 treatment increases amino acid uptake in jejunal preparations from mice ex vivo [31]. This GLP-2-induced nutrient uptake, at least for amino acids, is due to increased expression of amino acid transporters within brush border membrane vesicles of enterocytes, the absorptive cells of the gut [31]. In patients with short-bowel syndrome, where the terminal ileum and colon have been resected, GLP-2 treatment improves nutrient absorption and nutritional status [26, 27].

GLP-2 also plays an important role in maintaining gut barrier function. High-fat diet (HFD) feeding increases gut permeability in rodents [8], which allows the gram-negative bacteria-derived endotoxin, lipopolysaccharide (LPS), to pass through the gut barrier into circulation. Elevated LPS levels contribute to the obesity-induced low-grade systemic inflammation seen in rodents and humans [8, 9]. Mice lacking functional GLP-2 receptor (GLP-2R) have increased abundances of gut microbial species, including *Firmicutes*, which are associated with worsened glucose homeostasis [32]. As well, *glp2r-/-* mice have increased intestinal expression of pro-inflammatory cytokines [32], suggesting a role for GLP-2 in helping to regulate the innate immune system in the gut. Similarly, GLP-2 treatment increases expression of tight junction proteins in the gut to improve gut barrier function and decrease gut permeability in HFD-fed mice [10]. Altogether, these studies highlight the importance of GLP-2 in maintaining gut homeostasis by interacting with multiple intestinal gut cell types and the microbiome. The beneficial intestinotrophic and absorptive properties of GLP-2 make it one of the most effective treatments for short bowel syndrome in humans. More preclinical studies are needed to fully determine whether the improvements in intestinal barrier function with GLP-2 can be translationally applied to treat metabolic disease.

9 The Gut Microbiome and Its Role in Obesity

Increasing evidence implicates the gut microbiome in contributing to host health and in the development of diet-induced obesity. Initial studies characterizing the microbiome signature of normal and obese rodents found that abundance levels of *Bacteroidetes* was reduced and *Firmicutes* was elevated in diet-induced and genetically obese rodents [81]. To support the causal role of the microbiome in the development of obesity, transplanting feces from obese rodents into normal lean mice caused body weight gain and increased adiposity in recipient mice [80]. Since these seminal studies, additional bacterial strains have been identified, including *Akkermansia muciniphila* and *Bifidobacteria*, all of which have a constellation of beneficial effects on improving host health and metabolism, including greater body weight loss, improved glucose homeostasis, decreased gut permeability and inflammation, and lowering of circulating lipids in rodents [30, 44, 56, 92]. Since the microbiome composition is largely determined by host diet, identifying gut species that associate with improved metabolic homeostasis may be therapeutically targeted or utilized as a supplement to treat diet-induced obesity and insulin resistance.

Gram negative bacteria generate lipopolysaccharide (LPS), a pro-inflammatory compound that is the natural ligand for Toll-like Receptor-4 (TLR4) [14, 62]. Plasma LPS levels are increased in obese rodents and humans, and this elevation is known as "metabolic endotoxemia" [8, 9]. Similarly, TLR4 expression is elevated in obese humans [1], which may be in response to elevated circulating LPS levels. Impaired gut barrier function promotes the development of metabolic disease by allowing LPS to enter into circulation and contribute to the low-grade inflammation seen in high fat diet-induced obesity [10, 39, 43]. Furthermore, the effects of LPS are seen in resident adipose tissue macrophages expressing TLR4 where under high fat diet conditions, these surveilling immune cells switch from an anti-inflammatory "M2-like" phenotype to a pro-inflammatory "M1-like" phenotype to contribute to systemic inflammation [69].

Next-generation sequencing and –omics analyses have made major advances to this growing field by moving beyond the descriptive characterization of the microbiome milieu to identify specific bacterial gene programs and their associated functional metabolites [41, 50, 60]. Microbe-derived metabolites, including serotonin and short-chain fatty acids, are synthesized in the gut and can enter circulation to impart important physiological effects [28, 48, 90]. For example, the short-chain fatty acid proprionate is generated by the mucin-degrading bacterium *Akkermansia* has beneficial metabolic effects in insulin resistant mice [16, 57, 71]. One mechanism by which SCFAs improve glucose homeostasis is by binding to G-protein coupled receptors, including GPR41 and GPR43 on enteroendocrine cells, to augment GLP-1 secretion [79]. Similarly, feeding insulin resistant mice a diet high in soluble fiber increases intestinal gluconeogenesis as well as circulating levels of the short-chain fatty acids propionate and butyrate to subsequently improve glucose homeostasis in mice through a gut-brain pathway that is dependent on GPR41 [17].

Altogether, these studies highlight the translational potential of beneficially manipulating the gut microbiome through dietary supplementation of either the microbes directly or functional foods that are able to promote the synthesis of beneficial metabolites and/or microbial abundances that confer metabolic benefits to the host. Safety and tolerance for many of these potential microbial candidates remain to be validated in humans. However, pre-clinical studies and early-stage clinical trials demonstrate strong promise for using the gut microbiome as a therapeutic approach for improving metabolic homeostasis in humans. The dynamic responsivity of the gut microbiome to diet makes this symbiotic organ an attractive therapeutic target for treating obesity, Type 2 diabetes and Metabolic Syndrome in humans.

10 The Role of Intestinal Immune Cells in Obesity and Insulin Resistance

Innate and adaptive immune responses to luminal stimuli in the gut are critical for maintaining both intestinal and host homeostasis. Increasing evidence supports a role for aberrant intestinal immune responses in contributing to the development of diet-induced obese insulin resistance and metabolic disease. Intestinal immune cells reside either in localized immune areas such as Peyer's Patches and the lamina propria, or they are also diffusely located throughout the gut, such as gut intraepithelial T cells. While there is a diversity of immune cell types residing in the gut that are critical for protection against bacterial infection and viruses, T-lymphocytes (helper, regulatory and cytotoxic) are critical for moderating adaptive immune responses by secreting specific cytokine signals to recruit and/or aid other immune cell types to maintain host health. Aberrations in T-cell activation and downstream cytokine signaling cascades contribute to the pathophysiology of metabolic disease.

Mucosal integrity and gut barrier function are critical for maintaining intestinal homeostasis. High fat diet feeding impairs these architectural structures and contributes to LPS-induced metabolic endotoxemia and the development of insulin resistance and metabolic disease [9]. Genetic and diet-induced obese mice have impaired IL-22 response to bacterial infection, and treatment with IL-22 to obese mice was able to restore mucosal host defense to bacterial challenge. Furthermore, mice deficient in IL-22 receptor were more susceptible to diet-induced obesity and insulin resistance, and IL-22 treatment to genetically obese leptin receptor-deficient (*db/db*) mice fed high fat diet improved glucose tolerance and insulin resistance and restored the gut mucosal barrier [86].

The most abundant CD4 T+ cell sub-type in mucosal tissues is the Th17 cell type population [40, 93]. Th17 cells secrete IL-17 and/or IL-22, cytokines that are necessary for maintaining gut homeostasis [66, 86]. HFD impairs antigen-presenting cell activation to generate Th17 cells in vitro [20]. As well, colonizing germ-free mice with the ileal microbiome from HFD mice reduced the number of Th17 cells in lamina propria of recipient mice [20]. The importance of small intestinal Th17 cell

function in regulating glucose homeostasis is further supported by studies in RORγt-deficient mice lacking functional IL-17. RORγt-deficient mice fed chow diet gained more weight, were glucose intolerant and hypinsulinemic independent of food intake when compared to controls [20].

Immunoglobulin A (IgA) is a B cell antibody produced by resident plasma cells in the gut lamina propria and plays a critical role in commensal bacteria tolerance and protection of the gut mucosa from surface pathogens [12, 73, 85]. IgA is important for maintaining gut barrier function, yet its role in metabolic disease has only been more recently investigated. Colonic IgA levels are reduced in diet-induced obese mice, and HFD-fed mice lacking IgA have increased gut permeability, worsened insulin resistance and increased inflammation in peripheral metabolic tissues, including increased numbers of crown-like structures in white adipose tissue [43]. Furthermore, fecal transplantation studies in germ-free recipient mice demonstrate that the impaired glucose tolerance and insulin resistance observed in HFD-fed IgA-/- mice is a transmissible phenotype [43].

Altogether, these studies demonstrate the importance of gut immune cells in contributing to the regulation of glucose homeostasis. Furthermore, identifying gut immune targets with beneficial systemic effects to decrease inflammation and improve glucose homeostasis represents an exciting and novel approach to treat obesity and insulin resistance in humans.

11 Conclusions

Here we have highlighted key concepts and described relevant molecular players and their mechanisms of action that regulate immunometabolism and contribute to the pathogenesis of diet-induced obesity and Type 2 Diabetes. Despite major advances in determining causality of genes and/or molecular targets in metabolic disease, additional studies in preclinical models are needed to validate translational potential and long-term safety of these putative therapeutic applications to humans. Dysregulation of these molecular pathways with changes in lifestyle factors including exercise and diet demonstrates host responsiveness to its environment. Altogether, this review aims to introduce and intersect areas of immunology and metabolism that may drive newer areas of research to reveal novel insight and targets with which to maintain metabolic homeostasis and treat human disease.

References

1. Ahmad R, Al-Mass A, Atizado V, Al-Hubail A, Al-Ghimlas F, Al-Arouj M, Bennakhi A, Dermime S, Behbehani K (2012) Elevated expression of the toll like receptors 2 and 4 in obese individuals: its significance for obesity-induced inflammation. J Inflamm (Lond) 9(1):48. https://doi.org/10.1186/1476-9255-1189-1148

2. Arakawa M, Mita T, Azuma K, Ebato C, Goto H, Nomiyama T, Fujitani Y, Hirose T, Kawamori R, Watada H (2010) Inhibition of monocyte adhesion to endothelial cells and attenuation of atherosclerotic lesion by a glucagon-like peptide-1 receptor agonist, exendin-4. Diabetes 59(4):1030–1037
3. Astley CM, Todd JN, Salem RM, Vedantam S, Ebbeling CB, Huang PL, Ludwig DS, Hirschhorn JN, Florez JC (2018) Genetic evidence that carbohydrate-stimulated insulin secretion leads to obesity. Clin Chem 64(1):192–200
4. Benjamin EJ, Dupuis J, Larson MG, Lunetta KL, Booth SL, Govindaraju DR, Kathiresan S, Keaney JF Jr, Keyes MJ, Lin JP, Meigs JB, Robins SJ, Rong J, Schnabel R, Vita JA, Wang TJ, Wilson PW, Wolf PA, Vasan RS (2007) Genome-wide association with select biomarker traits in the Framingham Heart Study. BMC Med Genet 8(Suppl 1):S11
5. Berg AH, Lin Y, Lisanti MP, Scherer PE (2004) Adipocyte differentiation induces dynamic changes in NF-kappaB expression and activity. Am J Physiol Endocrinol Metab 287(6):E1178-1188
6. Boutens L, Hooiveld GJ, Dhingra S, Cramer RA, Netea MG, Stienstra R (2018) Unique metabolic activation of adipose tissue macrophages in obesity promotes inflammatory responses. Diabetologia 61(4):942–953
7. Bremer AA, Devaraj S, Afify A, Jialal I (2011) Adipose tissue dysregulation in patients with metabolic syndrome. J Clin Endocrinol Metab 96(11):E1782-1788
8. Cani PD, Amar J, Iglesias MA, Poggi M, Knauf C, Bastelica D, Neyrinck AM, Fava F, Tuohy KM, Chabo C, Waget A, Delmee E, Cousin B, Sulpice T, Chamontin B, Ferrieres J, Tanti JF, Gibson GR, Casteilla L, Delzenne NM, Alessi MC, Burcelin R (2007) Metabolic endotoxemia initiates obesity and insulin resistance. Diabetes 56(7):1761–1772
9. Cani PD, Bibiloni R, Knauf C, Waget A, Neyrinck AM, Delzenne NM, Burcelin R (2008) Changes in gut microbiota control metabolic endotoxemia-induced inflammation in high-fat diet-induced obesity and diabetes in mice. Diabetes 57(6):1470–1481
10. Cani PD, Possemiers S, Van de Wiele T, Guiot Y, Everard A, Rottier O, Geurts L, Naslain D, Neyrinck A, Lambert DM, Muccioli GG, Delzenne NM (2009) Changes in gut microbiota control inflammation in obese mice through a mechanism involving GLP-2-driven improvement of gut permeability. Gut 58(8):1091–1103
11. Castoldi A, Andrade-Oliveira V, Aguiar CF, Amano MT, Lee J, Miyagi MT, Latancia MT, Braga TT, da Silva MB, Ignacio A, Carola Correia Lima JD, Loures FV, Albuquerque JAT, Macedo MB, Almeida RR, Gaiarsa JW, Luevano-Martinez LA, Belchior T, Hiyane MI, Brown GD, Mori MA, Hoffmann C, Seelaender M, Festuccia WT, Moraes-Vieira PM, Camara NOS (2017). Dectin-1 activation exacerbates obesity and insulin resistance in the absence of MyD88. Cell Rep 19(11): 2272–2288
12. Chagwedera DN, Ang QY, Bisanz JE, Leong YA, Ganeshan K, Cai J, Patterson AD, Turnbaugh PJ, Chawla A (2019) Nutrient sensing in CD11c cells alters the gut microbiota to regulate food intake and body mass. Cell Metab 30(2):364-373.e367
13. Cho YM, Youn BS, Lee H, Lee N, Min SS, Kwak SH, Lee HK, Park KS (2006) Plasma retinol-binding protein-4 concentrations are elevated in human subjects with impaired glucose tolerance and type 2 diabetes. Diabetes Care 29(11):2457–2461
14. Chow JC, Young DW, Golenbock DT, Christ WJ, Gusovsky F (1999) Toll-like receptor-4 mediates lipopolysaccharide-induced signal transduction. J Biol Chem 274(16):10689–10692
15. Cinti S, Mitchell G, Barbatelli G, Murano I, Ceresi E, Faloia E, Wang S, Fortier M, Greenberg AS, Obin MS (2005) Adipocyte death defines macrophage localization and function in adipose tissue of obese mice and humans. J Lipid Res 46(11):2347–2355
16. Dao MC, Everard A, Aron-Wisnewsky J, Sokolovska N, Prifti E, Verger EO, Kayser BD, Levenez F, Chilloux J, Hoyles L, Dumas ME, Rizkalla SW, Dore J, Cani PD, Clement K (2016) Akkermansia muciniphila and improved metabolic health during a dietary intervention in obesity: relationship with gut microbiome richness and ecology. Gut 65(3):426–436. https://doi.org/10.1136/gutjnl-2014-308778. Epub 302015 Jun 308722
17. De Vadder F, Kovatcheva-Datchary P, Goncalves D, Vinera J, Zitoun C, Duchampt A, Backhed F, Mithieux G (2014) Microbiota-generated metabolites promote metabolic benefits via gut-brain neural circuits. Cell 156(1–2):84–96

18. Engstrom G, Hedblad B, Stavenow L, Lind P, Janzon L, Lindgarde F (2003) Inflammation-sensitive plasma proteins are associated with future weight gain. Diabetes 52(8):2097–2101
19. Frederich RC, Lollmann B, Hamann A, Napolitano-Rosen A, Kahn BB, Lowell BB, Flier JS (1995) Expression of ob mRNA and its encoded protein in rodents. Impact of nutrition and obesity. J Clin Invest 96(3):1658–1663
20. Garidou L, Pomie C, Klopp P, Waget A, Charpentier J, Aloulou M, Giry A, Serino M, Stenman L, Lahtinen S, Dray C, Iacovoni JS, Courtney M, Collet X, Amar J, Servant F, Lelouvier B, Valet P, Eberl G, Fazilleau N, Douin-Echinard V, Heymes C, Burcelin R (2015) The gut microbiota regulates intestinal CD4 T cells expressing RORgammat and controls metabolic disease. Cell Metab 22(1):100–112
21. Graham TE, Yang Q, Bluher M, Hammarstedt A, Ciaraldi TP, Henry RR, Wason CJ, Oberbach A, Jansson PA, Smith U, Kahn BB (2006) Retinol-binding protein 4 and insulin resistance in lean, obese, and diabetic subjects. N Engl J Med 354(24):2552–2563
22. Haider DG, Schindler K, Prager G, Bohdjalian A, Luger A, Wolzt M, Ludvik B (2007) Serum retinol-binding protein 4 is reduced after weight loss in morbidly obese subjects. J Clin Endocrinol Metab 92(3):1168–1171
23. Halaas JL, Gajiwala KS, Maffei M, Cohen SL, Chait BT, Rabinowitz D, Lallone RL, Burley SK, Friedman JM (1995) Weight-reducing effects of the plasma protein encoded by the obese gene. Science 269(5223):543–546
24. Hotamisligil GS, Arner P, Caro JF, Atkinson RL, Spiegelman BM (1995) Increased adipose tissue expression of tumor necrosis factor-alpha in human obesity and insulin resistance. J Clin Invest 95(5):2409–2415
25. Hotamisligil GS, Shargill NS, Spiegelman BM (1993) Adipose expression of tumor necrosis factor-alpha: direct role in obesity-linked insulin resistance. Science 259(5091):87–91
26. Jeppesen PB, Pertkiewicz M, Messing B, Iyer K, Seidner DL, J. O'Keefe S, Forbes A, Heinze H, Joelsson B (2012) Teduglutide reduces need for parenteral support among patients with short bowel syndrome with intestinal failure. Gastroenterology 143(6):1473–1481 e1473.
27. Jeppesen PB, Sanguinetti EL, Buchman A, Howard L, Scolapio JS, Ziegler TR, Gregory J, Tappenden KA, Holst J, Mortensen PB (2005) Teduglutide (ALX-0600), a dipeptidyl peptidase IV resistant glucagon-like peptide 2 analogue, improves intestinal function in short bowel syndrome patients. Gut 54(9):1224–1231
28. Ji J, Shu D, Zheng M, Wang J, Luo C, Wang Y, Guo F, Zou X, Lv X, Li Y, Liu T, Qu H (2016) Microbial metabolite butyrate facilitates M2 macrophage polarization and function. Sci Rep 6:24838
29. Lambert M, Delvin EE, Paradis G, O'Loughlin J, Hanley JA, Levy E (2004) C-reactive protein and features of the metabolic syndrome in a population-based sample of children and adolescents. Clin Chem 50(10):1762–1768
30. Le TK, Hosaka T, Nguyen TT, Kassu A, Dang TO, Tran HB, Pham TP, Tran QB, Le TH, Pham XD (2015) Bifidobacterium species lower serum glucose, increase expressions of insulin signaling proteins, and improve adipokine profile in diabetic mice. Biomed Res 36(1):63–70
31. Lee J, Koehler J, Yusta B, Bahrami J, Matthews D, Rafii M, Pencharz PB, Drucker DJ (2017) Enteroendocrine-derived glucagon-like peptide-2 controls intestinal amino acid transport. Mol Metab 6(3):245–255
32. Lee SJ, Lee J, Li KK, Holland D, Maughan H, Guttman DS, Yusta B, Drucker DJ (2012) Disruption of the murine Glp2r impairs Paneth cell function and increases susceptibility to small bowel enteritis. Endocrinology 153(3):1141–1151. 1110.1210/en.2011-1954. Epub 2012 Jan 1117
33. Lee YS, Jun HS (2016) Anti-inflammatory effects of GLP-1-based therapies beyond glucose control. Mediators Inflamm 2016:3094642
34. Lee YS, Park MS, Choung JS, Kim SS, Oh HH, Choi CS, Ha SY, Kang Y, Kim Y, Jun HS (2012) Glucagon-like peptide-1 inhibits adipose tissue macrophage infiltration and inflammation in an obese mouse model of diabetes. Diabetologia 55(9):2456–2468
35. Leong A, Chen J, Wheeler E, Hivert MF, Liu CT, Merino J, Dupuis J, Tai ES, Rotter JI, Florez JC, Barroso I, Meigs JB (2019) Mendelian randomization analysis of hemoglobin A1c as a risk factor for coronary artery disease. Diabetes Care 42(7):1202–1208

36. Ley RE, Backhed F, Turnbaugh P, Lozupone CA, Knight RD, Gordon JI (2005) Obesity alters gut microbial ecology. Proc Natl Acad Sci USA 102(31):11070–11075
37. Leyva F, Godsland IF, Ghatei M, Proudler AJ, Aldis S, Walton C, Bloom S, Stevenson JC (1998) Hyperleptinemia as a component of a metabolic syndrome of cardiovascular risk. Arterioscler Thromb Vasc Biol 18(6):928–933
38. Lim S, Choi SH, Jeong IK, Kim JH, Moon MK, Park KS, Lee HK, Kim YB, Jang HC (2008) Insulin-sensitizing effects of exercise on adiponectin and retinol-binding protein-4 concentrations in young and middle-aged women. J Clin Endocrinol Metab 93(6):2263–2268
39. Lin YH, Luck H, Khan S, Schneeberger PHH, Tsai S, Clemente-Casares X, Lei H, Leu YL, Chan YT, Chen HY, Yang SH, Coburn B, Winer S, Winer DA (2019) Aryl hydrocarbon receptor agonist indigo protects against obesity-related insulin resistance through modulation of intestinal and metabolic tissue immunity. Int J Obes (Lond).
40. Littman DR, Rudensky AY (2010) Th17 and regulatory T cells in mediating and restraining inflammation. Cell 140(6):845–858
41. Lloyd-Price J, Arze C, Ananthakrishnan AN, Schirmer M, Avila-Pacheco J, Poon TW, Andrews E, Ajami NJ, Bonham KS, Brislawn CJ, Casero D, Courtney H, Gonzalez A, Graeber TG, Hall AB, Lake K, Landers CJ, Mallick H, Plichta DR, Prasad M, Rahnavard G, Sauk J, Shungin D, Vazquez-Baeza Y, White RA 3rd, Braun J, Denson LA, Jansson JK, Knight R, Kugathasan S, McGovern DPB, Petrosino JF, Stappenbeck TS, Winter HS, Clish CB, Franzosa EA, Vlamakis H, Xavier RJ, Huttenhower C (2019) Multi-omics of the gut microbial ecosystem in inflammatory bowel diseases. Nature 569(7758):655–662
42. Lotta LA, Scott RA, Sharp SJ, Burgess S, Luan J, Tillin T, Schmidt AF, Imamura F, Stewart ID, Perry JR, Marney L, Koulman A, Karoly ED, Forouhi NG, Sjogren RJ, Naslund E, Zierath JR, Krook A, Savage DB, Griffin JL, Chaturvedi N, Hingorani AD, Khaw KT, Barroso I, McCarthy MI, O'Rahilly S, Wareham NJ, Langenberg C (2016) Genetic predisposition to an impaired metabolism of the branched-chain amino acids and risk of Type 2 diabetes: a mendelian randomisation analysis. PLoS Med 13(11):e1002179
43. Luck H, Khan S, Kim JH, Copeland JK, Revelo XS, Tsai S, Chakraborty M, Cheng K, Tao Chan Y, Nohr MK, Clemente-Casares X, Perry MC, Ghazarian M, Lei H, Lin YH, Coburn B, Okrainec A, Jackson T, Poutanen S, Gaisano H, Allard JP, Guttman DS, Conner ME, Winer S, Winer DA (2019) Gut-associated IgA(+) immune cells regulate obesity-related insulin resistance. Nat Commun 10(1):3650
44. Lukovac S, Belzer C, Pellis L, Keijser BJ, de Vos WM, Montijn RC, Roeselers G (2014) Differential modulation by Akkermansia muciniphila and Faecalibacterium prausnitzii of host peripheral lipid metabolism and histone acetylation in mouse gut organoids. MBio 5(4)
45. Lynch L, Hogan AE, Duquette D, Lester C, Banks A, LeClair K, Cohen DE, Ghosh A, Lu B, Corrigan M, Stevanovic D, Maratos-Flier E, Drucker DJ, O'Shea D, Brenner M (2016) iNKT cells induce FGF21 for thermogenesis and are required for maximal weight loss in GLP1 therapy. Cell Metab 24(3):510–519
46. Lynch L, Michelet X, Zhang S, Brennan PJ, Moseman A, Lester C, Besra G, Vomhof-Dekrey EE, Tighe M, Koay HF, Godfrey DI, Leadbetter EA, Sant'Angelo DB, von Andrian U, Brenner MB (2015) Regulatory iNKT cells lack expression of the transcription factor PLZF and control the homeostasis of T(reg) cells and macrophages in adipose tissue. Nat Immunol 16(1):85–95
47. Lynch L, Nowak M, Varghese B, Clark J, Hogan AE, Toxavidis V, Balk SP, O'Shea D, O'Farrelly C, Exley MA (2012) Adipose tissue invariant NKT cells protect against diet-induced obesity and metabolic disorder through regulatory cytokine production. Immunity 37(3):574–587
48. Macia L, Tan J, Vieira AT, Leach K, Stanley D, Luong S, Maruya M, Ian McKenzie C, Hijikata A, Wong C, Binge L, Thorburn AN, Chevalier N, Ang C, Marino E, Robert R, Offermanns S, Teixeira MM, Moore RJ, Flavell RA, Fagarasan S, Mackay CR (2015) Metabolite-sensing receptors GPR43 and GPR109A facilitate dietary fibre-induced gut homeostasis through regulation of the inflammasome. Nat Commun 6:6734
49. Maffei M, Fei H, Lee GH, Dani C, Leroy P, Zhang Y, Proenca R, Negrel R, Ailhaud G, Friedman JM (1995) Increased expression in adipocytes of ob RNA in mice with lesions of the hypothalamus and with mutations at the db locus. Proc Natl Acad Sci USA 92(15):6957–6960

50. Mallick H, Franzosa EA, McLver LJ, Banerjee S, Sirota-Madi A, Kostic AD, Clish CB, Vlamakis H, Xavier RJ, Huttenhower C (2019) Predictive metabolomic profiling of microbial communities using amplicon or metagenomic sequences. Nat Commun 10(1):3136
51. Martin GR, Wallace LE, Hartmann B, Holst JJ, Demchyshyn L, Toney K, Sigalet DL (2005) Nutrient-stimulated GLP-2 release and crypt cell proliferation in experimental short bowel syndrome. Am J Physiol Gastrointest Liver Physiol 288(3):G431-438
52. Mehta NN, McGillicuddy FC, Anderson PD, Hinkle CC, Shah R, Pruscino L, Tabita-Martinez J, Sellers KF, Rickels MR, Reilly MP (2010) Experimental endotoxemia induces adipose inflammation and insulin resistance in humans. Diabetes 59(1):172–181
53. Meier JJ, Nauck MA, Pott A, Heinze K, Goetze O, Bulut K, Schmidt WE, Gallwitz B, Holst JJ (2006) Glucagon-like peptide 2 stimulates glucagon secretion, enhances lipid absorption, and inhibits gastric acid secretion in humans. Gastroenterology 130(1):44–54
54. Moraes-Vieira PM, Larocca RA, Bassi EJ, Peron JP, Andrade-Oliveira V, Wasinski F, Araujo R, Thornley T, Quintana FJ, Basso AS, Strom TB, Camara NO (2014) Leptin deficiency impairs maturation of dendritic cells and enhances induction of regulatory T and Th17 cells. Eur J Immunol 44(3):794–806
55. Moraes-Vieira PM, Yore MM, Dwyer PM, Syed I, Aryal P, Kahn BB (2014) RBP4 activates antigen-presenting cells, leading to adipose tissue inflammation and systemic insulin resistance. Cell Metab 19(3):512–526
56. Moya-Perez A, Neef A, Sanz Y (2015) Bifidobacterium pseudocatenulatum CECT 7765 reduces obesity-associated inflammation by restoring the lymphocyte-macrophage balance and gut microbiota structure in high-fat diet-fed mice. PLoS One. 10(7):e0126976. 0126910.0121371/journal.pone.0126976. eCollection 0122015
57. Mujico JR, Baccan GC, Gheorghe A, Diaz LE, Marcos A, Underwood MA, Tilg H, Moschen AR, Arslan N, Hamilton MK, Boudry G, Lemay DG, Raybould HE, Zhang C, Yin A, Li H, Wang R, Wu G, Shen J, Zhang M, Wang L, Hou Y, Ouyang H, Zhang Y, Zheng Y, Wang J, Lv X, Wang Y, Zhang F, Zeng B, Li W, Yan F, Zhao Y, Pang X, Zhang X, Fu H, Chen F, Zhao N, Hamaker BR, Bridgewater LC, Weinkove D, Clement K, Dore J, Holmes E, Xiao H, Zhao G, Yang S, Bork P, Nicholson JK, Wei H, Tang H, Zhao L, Moya-Perez A, Neef A, Sanz Y, Henao-Mejia J, Elinav E, Jin C, Hao L, Mehal WZ, Strowig T, Thaiss CA, Kau AL, Eisenbarth SC, Jurczak MJ, Camporez JP, Shulman GI, Gordon JI, Hoffman HM, Flavell RA (2013) Changes in gut microbiota due to supplemented fatty acids in diet-induced obese mice. Intestinal dysbiosis: novel mechanisms by which gut microbes trigger and prevent disease. Microbiota and diabetes: an evolving relationship. Obesity, fatty liver disease and intestinal microbiota. Changes in intestinal barrier function and gut microbiota in high-fat diet-fed rats are dynamic and region dependent. Dietary modulation of gut microbiota contributes to alleviation of both genetic and simple obesity in children. Bifidobacterium pseudocatenulatum CECT 7765 Reduces obesity-associated inflammation by restoring the lymphocyte-macrophage balance and gut microbiota structure in high-fat diet-fed mice. Inflammasome-mediated dysbiosis regulates progression of NAFLD and obesity. Br J Nutr 110(4):711–720. https://doi.org/10.1017/S0007114512005612 Epub 0007114512002013 Jan 0007114512005610
58. Murano I, Barbatelli G, Parisani V, Latini C, Muzzonigro G, Castellucci M, Cinti S (2008) Dead adipocytes, detected as crown-like structures, are prevalent in visceral fat depots of genetically obese mice. J Lipid Res 49(7):1562–1568
59. Nicholas DA, Proctor EA, Agrawal M, Belkina AC, Van Nostrand SC, Panneerseelan-Bharath L, Jones ARt, Raval F, Ip BC, Zhu M, Cacicedo JM, Habib C, Sainz-Rueda N, Persky L, Sullivan PG, Corkey BE, Apovian CM, Kern PA, Lauffenburger DA, Nikolajczyk BS (2019) Fatty acid metabolites combine with reduced beta oxidation to activate Th17 inflammation in human Type 2 diabetes. Cell Metab 30(3): 447–461.e445
60. Pasolli E, Asnicar F, Manara S, Zolfo M, Karcher N, Armanini F, Beghini F, Manghi P, Tett A, Ghensi P, Collado MC, Rice BL, DuLong C, Morgan XC, Golden CD, Quince C, Huttenhower C, Segata N (2019) Extensive unexplored human microbiome diversity revealed by over 150,000 genomes from metagenomes spanning age, geography, and lifestyle. Cell 176(3):649-662.e620

61. Peraldi P, Hotamisligil GS, Buurman WA, White MF, Spiegelman BM (1996) Tumor necrosis factor (TNF)-alpha inhibits insulin signaling through stimulation of the p55 TNF receptor and activation of sphingomyelinase. J Biol Chem 271(22):13018–13022
62. Poltorak A, He X, Smirnova I, Liu MY, Van Huffel C, Du X, Birdwell D, Alejos E, Silva M, Galanos C, Freudenberg M, Ricciardi-Castagnoli P, Layton B, Beutler B (1998) Defective LPS signaling in C3H/HeJ and C57BL/10ScCr mice: mutations in Tlr4 gene. Science 282(5396):2085–2088
63. Qin J, Li Y, Cai Z, Li S, Zhu J, Zhang F, Liang S, Zhang W, Guan Y, Shen D, Peng Y, Zhang D, Jie Z, Wu W, Qin Y, Xue W, Li J, Han L, Lu D, Wu P, Dai Y, Sun X, Li Z, Tang A, Zhong S, Li X, Chen W, Xu R, Wang M, Feng Q, Gong M, Yu J, Zhang Y, Zhang M, Hansen T, Sanchez G, Raes J, Falony G, Okuda S, Almeida M, LeChatelier E, Renault P, Pons N, Batto JM, Zhang Z, Chen H, Yang R, Zheng W, Li S, Yang H, Wang J, Ehrlich SD, Nielsen R, Pedersen O, Kristiansen K, Wang J (2012) A metagenome-wide association study of gut microbiota in type 2 diabetes. Nature 490(7418):55–60
64. Roh HC, Tsai LT, Lyubetskaya A, Tenen D, Kumari M, Rosen ED (2017) Simultaneous transcriptional and epigenomic profiling from specific cell types within heterogeneous tissues in vivo. Cell Rep 18(4):1048–1061
65. Rong R, Hanson RL, Ortiz D, Wiedrich C, Kobes S, Knowler WC, Bogardus C, Baier LJ (2009) Association analysis of variation in/near FTO, CDKAL1, SLC30A8, HHEX, EXT2, IGF2BP2, LOC387761, and CDKN2B with type 2 diabetes and related quantitative traits in Pima Indians. Diabetes 58(2):478–488
66. Rovedatti L, Kudo T, Biancheri P, Sarra M, Knowles CH, Rampton DS, Corazza GR, Monteleone G, Di Sabatino A, Macdonald TT (2009) Differential regulation of interleukin 17 and interferon gamma production in inflammatory bowel disease. Gut 58(12):1629–1636
67. Saxena R, Voight BF, Lyssenko V, Burtt NP, de Bakker PI, Chen H, Roix JJ, Kathiresan S, Hirschhorn JN, Daly MJ, Hughes TE, Groop L, Altshuler D, Almgren P, Florez JC, Meyer J, Ardlie K, Bengtsson Bostrom K, Isomaa B, Lettre G, Lindblad U, Lyon HN, Melander O, Newton-Cheh C, Nilsson P, Orho-Melander M, Rastam L, Speliotes EK, Taskinen MR, Tuomi T, Guiducci C, Berglund A, Carlson J, Gianniny L, Hackett R, Hall L, Holmkvist J, Laurila E, Sjogren M, Sterner M, Surti A, Svensson M, Svensson M, Tewhey R, Blumenstiel B, Parkin M, Defelice M, Barry R, Brodeur W, Camarata J, Chia N, Fava M, Gibbons J, Handsaker B, Healy C, Nguyen K, Gates C, Sougnez C, Gage D, Nizzari M, Gabriel SB, Chirn GW, Ma Q, Parikh H, Richardson D, Ricke D, Purcell S (2007) Genome-wide association analysis identifies loci for type 2 diabetes and triglyceride levels. Science 316(5829):1331–1336
68. Sewter CP, Digby JE, Blows F, Prins J, O'Rahilly S (1999) Regulation of tumour necrosis factor-alpha release from human adipose tissue in vitro. J Endocrinol 163(1):33–38
69. Shaul ME, Bennett G, Strissel KJ, Greenberg AS, Obin MS (2010) Dynamic, M2-like remodeling phenotypes of CD11c+ adipose tissue macrophages during high-fat diet–induced obesity in mice. Diabetes 59(5):1171–1181
70. Shi H, Kokoeva MV, Inouye K, Tzameli I, Yin H, Flier JS (2006) TLR4 links innate immunity and fatty acid-induced insulin resistance. J Clin Invest 116(11):3015–3025
71. Shin NR, Lee JC, Lee HY, Kim MS, Whon TW, Lee MS, Bae JW (2014) An increase in the Akkermansia spp. population induced by metformin treatment improves glucose homeostasis in diet-induced obese mice. Gut 63(5):727–735
72. Sjoholm A, Nystrom T (2005) Endothelial inflammation in insulin resistance. Lancet 365(9459):610–612
73. Soltoft J, Soeberg B (1972) Immunoglobulin-containing cells in the small intestine during acute enteritis. Gut 13(7):535–538
74. Strissel KJ, Stancheva Z, Miyoshi H, Perfield JW 2nd, DeFuria J, Jick Z, Greenberg AS, Obin MS (2007) Adipocyte death, adipose tissue remodeling, and obesity complications. Diabetes 56(12):2910–2918
75. Stumvoll M, Tschritter O, Fritsche A, Staiger H, Renn W, Weisser M, Machicao F, Haring H (2002) Association of the T-G polymorphism in adiponectin (exon 2) with obesity and insulin sensitivity: interaction with family history of type 2 diabetes. Diabetes 51(1):37–41

76. Tannahill GM, Curtis AM, Adamik J, Palsson-McDermott EM, McGettrick AF, Goel G, Frezza C, Bernard NJ, Kelly B, Foley NH, Zheng L, Gardet A, Tong Z, Jany SS, Corr SC, Haneklaus M, Caffrey BE, Pierce K, Walmsley S, Beasley FC, Cummins E, Nizet V, Whyte M, Taylor CT, Lin H, Masters SL, Gottlieb E, Kelly VP, Clish C, Auron PE, Xavier RJ, O'Neill LA (2013) Succinate is an inflammatory signal that induces IL-1beta through HIF-1alpha. Nature 496(7444):238–242
77. Tannahill GM, O'Neill LA (2011) The emerging role of metabolic regulation in the functioning of Toll-like receptors and the NOD-like receptor Nlrp3. FEBS Lett 585(11):1568–1572
78. Tashiro Y, Sato K, Watanabe T, Nohtomi K, Terasaki M, Nagashima M, Hirano T (2014) A glucagon-like peptide-1 analog liraglutide suppresses macrophage foam cell formation and atherosclerosis. Peptides 54:19–26
79. Tolhurst G, Heffron H, Lam YS, Parker HE, Habib AM, Diakogiannaki E, Cameron J, Grosse J, Reimann F, Gribble FM (2012) Short-chain fatty acids stimulate glucagon-like peptide-1 secretion via the G-protein-coupled receptor FFAR2. Diabetes 61(2):364–371
80. Turnbaugh PJ, Backhed F, Fulton L, Gordon JI (2008) Diet-induced obesity is linked to marked but reversible alterations in the mouse distal gut microbiome. Cell Host Microbe 3(4):213–223
81. Turnbaugh PJ, Ley RE, Mahowald MA, Magrini V, Mardis ER, Gordon JI (2006) An obesity-associated gut microbiome with increased capacity for energy harvest. Nature 444(7122):1027–1031
82. Uysal KT, Wiesbrock SM, Marino MW, Hotamisligil GS (1997) Protection from obesity-induced insulin resistance in mice lacking TNF-alpha function. Nature 389(6651):610–614
83. Visser M, Bouter LM, McQuillan GM, Wener MH, Harris TB (1999) Elevated C-reactive protein levels in overweight and obese adults. JAMA 282(22):2131–2135
84. Vitseva OI, Tanriverdi K, Tchkonia TT, Kirkland JL, McDonnell ME, Apovian CM, Freedman J, Gokce N (2008) Inducible Toll-like receptor and NF-kappaB regulatory pathway expression in human adipose tissue. Obesity (Silver Spring) 16(5):932–937
85. Vulevic J, Juric A, Tzortzis G, Gibson GR (2013) A mixture of trans-galactooligosaccharides reduces markers of metabolic syndrome and modulates the fecal microbiota and immune function of overweight adults. J Nutr 143(3):324–331
86. Wang X, Ota N, Manzanillo P, Kates L, Zavala-Solorio J, Eidenschenk C, Zhang J, Lesch J, Lee WP, Ross J, Diehl L, van Bruggen N, Kolumam G, Ouyang W (2014) Interleukin-22 alleviates metabolic disorders and restores mucosal immunity in diabetes. Nature 514(7521):237–241
87. Weisberg SP, McCann D, Desai M, Rosenbaum M, Leibel RL, Ferrante AW Jr (2003) Obesity is associated with macrophage accumulation in adipose tissue. J Clin Invest 112(12):1796–1808
88. Weyer C, Yudkin JS, Stehouwer CD, Schalkwijk CG, Pratley RE, Tataranni PA (2002) Humoral markers of inflammation and endothelial dysfunction in relation to adiposity and in vivo insulin action in Pima Indians. Atherosclerosis 161(1):233–242
89. Yang Q, Graham TE, Mody N, Preitner F, Peroni OD, Zabolotny JM, Kotani K, Quadro L, Kahn BB (2005) Serum retinol binding protein 4 contributes to insulin resistance in obesity and type 2 diabetes. Nature 436(7049):356–362
90. Yano JM, Yu K, Donaldson GP, Shastri GG, Ann P, Ma L, Nagler CR, Ismagilov RF, Mazmanian SK, Hsiao EY (2015) Indigenous bacteria from the gut microbiota regulate host serotonin biosynthesis. Cell 161(2):264–276
91. Yusta B, Baggio LL, Koehler J, Holland D, Cao X, Pinnell LJ, Johnson-Henry KC, Yeung W, Surette MG, Bang KW, Sherman PM, Drucker DJ (2015) GLP-1R agonists modulate enteric immune responses through the intestinal intraepithelial lymphocyte GLP-1R. Diabetes 64(7):2537–2549
92. Zhang C, Yin A, Li H, Wang R, Wu G, Shen J, Zhang M, Wang L, Hou Y, Ouyang H, Zhang Y, Zheng Y, Wang J, Lv X, Wang Y, Zhang F, Zeng B, Li W, Yan F, Zhao Y, Pang X, Zhang X, Fu H, Chen F, Zhao N, Hamaker BR, Bridgewater LC, Weinkove D, Clement K, Dore J, Holmes E, Xiao H, Zhao G, Yang S, Bork P, Nicholson JK, Wei H, Tang H, Zhao L (2015) Dietary modulation of gut microbiota contributes to alleviation of both genetic and simple obesity in children. EBioMedicine 2(8):968–984. https://doi.org/10.1016/j.ebiom.2015.1007.1007. eCollection 2015 Aug

93. Zhou L, Lopes JE, Chong MM, Ivanov R II, Min GD, Victora Y, Shen J, Du YP, Rubtsov AY, Rudensky SF, Ziegler and D. R. Littman, (2008) TGF-beta-induced Foxp3 inhibits T(H)17 cell differentiation by antagonizing RORgammat function. Nature 453(7192):236–240

Metabolic Reprogramming and Infectious Diseases

Érika Machado de Salles, Bárbara Cristina Pizzolante, and Denise Morais da Fonseca

Abbreviations

ACL	ATP-dependent Citrate Lyase
AMPK	AMP-activated protein kinase
AMPs	Antimicrobial peptides
AMs	Alveolar macrophages
Arg -1	Arginase-1
ASN	Asparagine
BCG	Baccillus Calmette-Guérin
COVID-19	Coronavirus disease 2019
DCs	Dendritic cells
FAS	Fatty Acid Synthesis
GALT	Gut-Associated Lymphoid Tissue
GAS	Group A Streptococcus
GLUT1	Glucose Transporter 1
HIF	αHypoxia-Inducible Factor 1-Alpha
HK2	Hexokinase 2
IBPs	Intracellular Bacterial Pathogens
IFNs	Interferons
IgA	Immunoglobulin A
IgG	Immunoglobulin G
IL	Interleukin

É. M. de Salles
Laboratory of Immunology of Infectious Diseases, Department of Immunology, Institute of Biomedical Sciences, University of Sao Paulo, Sao Paulo, Brazil

B. C. Pizzolante · D. M. da Fonseca (✉)
Laboratory of Mucosal Immunology, Department of Immunology, Institute of Biomedical Sciences, University of Sao Paulo, Sao Paulo, Brazil
e-mail: denisefonseca@usp.br

N. O. S. Camara et al. (eds.), *Essential Aspects of Immunometabolism in Health and Disease*, https://doi.org/10.1007/978-3-030-86684-6_8

ILCs	Innate lymphoid cells
ILFs	Isolated Lymphoid Follicles
JE	Junctional epithelium
LPS	Lipopolysaccharide
MALT	Mucosa-associated lymphoid tissue
MHC	Major Histocompatibility Complex
MIF	Migration Inhibitory Factor
MS	Multiple Sclerosis
mTOR	Mammalian Target of Rapamycin
mTORC1	Mammalian Target of Rapamycin complex 1
MV	Measles Virus
NETs	Neutrophil Extracellular Traps
NK	Natural killer
NLRP3	NLR family pyrin domain containing 3
NO	Nitric Oxide
Nrf2	Nuclear Factor erythroid-derived 2
OAA	Oxaloacetate
OXPHOS	Oxidative Phosphorylation
PAMPs	PathogenAssociated Molecular Patterns
PDK1	Phosphoinositide-Dependent Kinase 1
PFK1	Phosphofructokinase 1
PGE2	Prostaglandin E2
PI3K	Phosphoinositide-3 Kinase
pIgA	Polymeric Immunoglobulin A
pIgR	Polymeric immunoglobulin receptor
PIP3	Phosphatidylinositol-3,4,5-Trisphosphate
PKM2	Pyruvate Kinase M2
PPARδ	Peroxisome Proliferator-Activated Receptor Gamma
PPP	Pentose Phosphate Pathway
PPs	Peyer's Patches
PRRs	Pattern Recognition Receptors
ROS	Reactive Oxygen Species
SARS-CoV-2	Respiratory Syndrome Coronavirus 2
SH2	Src Homology 2
TAC	Tricarboxylic Acid Cycle
TGF-β	Transforming growth factor-β
Th	T helper
TLRs	Toll-like receptors
Tr1	Type 1 Regulatory T cells
Tregs	Regulatory T cells
TRMs	Tissue-Resident Macrophages
UTIs	Urinary tract infections
α-KG	α-Ketoglutarate

1 Barrier Tissues as Major Sites for Pathogen Entrance

Barrier tissues are in close contact with the external environment and exposed to a variety of antigens. These sites are endowed with characteristics that differentiate them from the other tissues due to their protective mechanisms against external agents. Such functions place the barrier tissues as the first line of host response to changes in homeostasis, including skin, oral cavity, respiratory, genitourinary and gastrointestinal tracts. Since these sites are highly exposed to environmental antigens, commensal microbes and pathogens, a selective barrier is crucial to limit the entry of external contents to the organism. The firewall mechanisms include cells and their products that act physically or actively to protect the organism. Besides, the barrier-associated immune system promotes effective immune surveillance, preventing exacerbated inflammatory responses, as well as tolerating commensal and innocuous antigens [8]. Next, we describe the barrier components that contribute to tissue-specific immunity and how episodes of infection could influence the metabolic reprogramming of immune cells.

The different tissues comprising the host barriers share structural components such as epithelial cells connected by tight junctions controlling tissue permeability, antimicrobial peptide-secreting cells, antibody-secreting B cells and other specialized epithelial cells supporting the chemical barrier. Also, a full network of immune cells covering mononuclear phagocytes, innate and adaptive lymphocytes, is in charge of monitoring the barriers. According to the organization of such components, the mucosal tissues are classified into two types: I and II (Table 1). Type I mucosal surfaces are covered by a single columnar epithelium, as seen in the small and large intestine, pseudostratified epithelia of the respiratory tract and the female upper reproductive tract. Common features comprise mucus-secreting goblet cells and polymeric immunoglobulin receptor (pIgR) expression at the basolateral surface. The pIgR binds to polymeric Immunoglobulin A (pIgA) secreted by plasma cells and releases the secretory IgA into the lumen. Hence, IgA is the main protective antibody in type I mucosal surfaces and contributes to the immune firewall by neutralizing

Table 1 Immune composition of barrier tissues and products that assist in the defense of the host

Components	Type I mucosa	Type II mucosa
Tissues	Intestine, respiratory tract, upper female reproductive tract	Vagina, eyes, mouth
Epithelia	Simple columnar	Stratified squamous
Mucus source	Goblet cells	Epithelial cells
IgA transport	Present	Absent
Major antibody isotype	Secretory IgA	Absent
Submucosa cells	DCs, macrophages, memory lymphocyte	Sparse network of DCs, macrophages, rare lymphocytes

Abbreviations Ig, Immunoglobulin; MALT, Mucosa Associated Lymphoid Tissues; DCs, dendritic cells

toxins and microbial-associated antigens [45]. Innate and adaptive immune cells in the type I mucosal tissues are classically found in the mucosa-associated lymphoid tissue (MALT), while type II mucosal surfaces are characterized by sparse clusters of immune cells in the submucosa. Type II mucosal surfaces, including the lower female reproductive tract, eyes and mouth, are characterized by a protective stratified squamous epithelial layer (keratinocytes), which shares many features with the skin. However, type II mucosal surfaces are protected by mucous and usually do not undergo terminal cornification. Also, the absence of pIgR in keratinocytes from stratified epithelial impairs IgA secretion into the lumen. Therefore, IgG is the main antibody found on the type II mucosa [44]. Despite the similarities among the firewall mechanisms, each barrier has unique characteristics providing the immune tone of tissue-specific responses, as depicted below.

1.1 Skin Barrier

In order to prevent physical insults and pathogen entrance, the body is covered by a keratinized stratified epithelium comprising the epidermis and dermis. Immunocompetent cells are located in both compartments, and the dead cells compose the stratum corneum that forms the outermost layer protecting the skin from injuries and infection. The skin surface holds a variety of microbial communities that shapes the immunological tone of the tissue [7, 69]. By controlling the expression of innate immune components by keratinocytes, e. g. cathelicidins, β-defensins and other antimicrobial peptides (AMPs), the skin-resident microbiota protects the host from the colonization by pathogenic species [32, 66]. Also, the skin commensals modulate the production of IL-17A and IFN-γ by local T cells that are involved in both host defense and inflammatory diseases [68, 69]. Through the expression of Toll-like receptors (TLRs), keratinocytes favor the differentiation of CD4 T lymphocytes to the T helper (Th) 1 profile and the production of interferons (IFNs), IL-1β, IL-18 and IL-6 that coordinate leukocyte traffic to the skin [60]. A specialized subset of mononuclear phagocyte, the Langerhans cells, projects the dendrites towards the outermost layers to uptake antigens and stimulate the adaptive immune response. In the dermis, mast cells store large amounts of substances in granules, such as cathelicidins, histamine and proteinases, that contribute to resident dendritic cell maturation and the immune dialogue with T cells [94].

1.2 Airway Barrier

Human lungs consume up to 11, 500L of air each day, therefore exposed to an enormous amount of particulates, as dust, smoke, pollution, pollen and aerosols [86]. The immune system associated with the airways ensures that inappropriate responses are not initiated against such particulate innocuous antigens, preventing

tissue damage and inflammatory disorders [5, 86]. However, lung immunity also needs to be effective due to the massive surface of the epithelial area in contract with the environment and the intimate association of an extensive blood capillaries network, which turns the respiratory tract into a major portal for pathogen entrance.

The continuous rhythmic movements of the cilia and constant production of a thin mucous layer by goblet cells afford the first barrier protection by trapping airborne particles and removing them out of the lung. The immune system associated with the respiratory tract consists of a specialized network of lung-resident innate cells, comprising epithelial cells, macrophages, dendritic cells and innate lymphoid cells (mostly from type 1 and 2 subsets), interacting with local stromal cells. Alveolar macrophages (AMs) are thought to account for 90–95% of lung immune cells during homeostatic conditions and are critical for the phagocytosis of invading microorganisms and also for the production of transforming growth factor-β (TGF-β) to maintain the homeostasis [5, 110]. AMs also phagocytes particulate matter, dying cells and cellular debris, limiting lung inflammation and promoting tissue repair after the clearance of infection. In addition to TGF-β, retinoic acid metabolized by AMs can convert naïve or activated T cells into regulatory T cells [96], crucial to control immune responses against lung disease. Pathogens that evade the aforementioned innate mechanisms of immunity require the activation of T cell subsets for the ultimate control of infection, and DCs are crucial for stimulation and differentiation of specialized T cell subsets. In this context, surveillance of the lung environment and uptake of foreign airborne particles are controlled by lung-resident bronchial, interstitial, and alveolar macrophages, as well as by conventional, plasmacytoid, and monocyte-derived dendritic cells.

1.3 Urinary Tract Barrier

The urinary tract consists of the kidneys, bladder, ureters and urethra, and, except for the urethra, most of it is considered to be a sterile microenvironment. In addition to the anatomical barrier and mucus layer [36], as protective mechanisms, the urinary tract has soluble components secreted in the urine, such as the glycoprotein plaque uroplatins, which protect against microbial colonization. [109]. Also, epithelial and immune cells residing in the urinary tract reinforce protection against infections that, in the urinary tract, are mainly caused by intestinal microorganisms. The epithelial cells secrete several soluble components, such as the cytokines IL-1, IL-6 [95] and IL-8 [67], promoting the enrollment of phagocytes to the bladder or kidney [2], uromodulin prevents interactions between pathogens and epithelial cells and antimicrobial factors inhibit microbial growth, e. g. neutrophil gelatinase-associated lipocalin, AMPs, and pentraxins. In the early stage of urinary tract infections (UTIs), neutrophils are recruited into the bladder to promote the bacterial clearance [38]. Resident macrophages in the bladder act as sentinels secreting the chemokines such as CXC-chemokine ligand 1 and 2 and macrophage migration inhibitory factor (MIF) to recruit and stimulates transepithelial movement of neutrophils and other immune

cells to combat the pathogens [88]. Mast cells also play a pivotal sentinel role during UTIs, due to the release of pre-stored proinflammatory mediators such as TNF, histamine and several chemokines [1]. During the resolution phase of infection, mast cells secrete IL-10 that have been associated with the regeneration of the bladder epithelium [17]. Of note, while the innate cells of the urinary tract are highly reactive in the presence of microbes, the adaptive immunity, particularly in the bladder, tends to be limited. In the context of adaptive immunity, the arrival of the infectious agent in the kidneys leads to the production of specific antibodies but a robust activation of adaptive immunity is not seen in the bladder [55, 79]. The limited ability of the bladder-associated immune system to become activated and differentiate into memory cells could be a main cause for the return of UTIs, which may be linked to the increase of local IL-10 production by mast cells.

1.4 Oral and Intestinal Barriers

The oral mucosa is lined by non-keratinized stratified squamous epithelia, while regions with mechanical stimulation or mastication injury have a protective layer of keratin. The junctional epithelium (JE) that lines the teeth is an extremely vulnerable site in the oral barrier. The JE connection to the tooth is highly permeable to the passage of fluid containing host protective factors, including plasma proteins, cytokines, immunoglobulins, and cells. Notably, the neutrophils are the main cells recruited to the gingival crevice and form a "defense wall" in the subgingival biofilm. [22]. In parallel, the gingival mononuclear phagocyte set comprises a complex network of DCs, macrophages, and recruited monocytes. Particularly, macrophages mediate the main antimicrobial functions at the gingiva but can also participate in healing and continuous tissue repair [57]. In the gingiva, also reside innate lymphoid cells (ILC), natural killer (NK) cells, B cells and T helper 17 (Th17) cells. Recently, it has been demonstrated that Th17 cells in the gingiva increase with age in response to local damage from chewing. [26]. IL-17 produced by Th17 cells is crucial for innate antimicrobial defense mechanisms, such as β-defensin 3, crucial for oral antifungal immunity. In addition to immune cells, saliva also comprises a plethora of antimicrobial agents, such as immunoglobulins, lysozyme, lactoferrin and peroxidases [42].

Moving forward towards the gastrointestinal tract, the intestine is specifically adapted to support (1) the colonization of commensal microorganisms, essential to promote the digestion and absorption of dietary nutrients, (2) tolerance towards innocuous environmental antigens and (3) immunity against infectious challenges. Notably, the gut epithelial and immune cells detect the microbiota components and its metabolites to maintain the tone of the tissue-specific immune response (Fig. 1). The primary firewall preventing pathogen and pathobiont invasion in the gut is the mucus layer. Ranging from a thin to a thick layer along the gastrointestinal mucosa, this multi-component structure integrates an intricated network of mucin glycoproteins along with the presence of AMPs and neutralizing IgA antibodies. Underneath

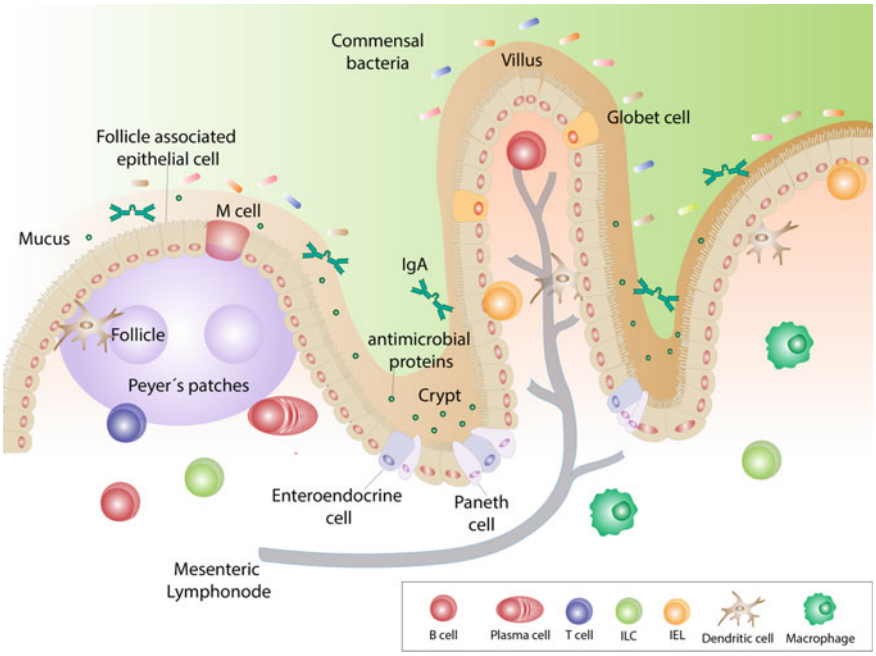


Fig. 1 The intestinal barrier comprises physical, chemical and active structures. The physical barrier includes the epithelium and a mucus layer. Along with epithelial cells, other cell subtypes contribute to the defense of the mucosa. The antimicrobial peptides and the immunoglobulin A (IgA) provide chemical strength to prevent the pathogen invasion and the host immune cells act as an active barrier, controlling the homeostasis and responding to invading microorganisms

the mucus layer, the intestinal lamina propria is covered by an epithelial cell monolayer. The intestinal epithelium is organized into crypts and villi, being the crypt compartment organized as invagination in the underlying lamina propria and responsible for the epithelium regeneration and microbicidal activity by AMPs-producing Paneth cells [76]. Among the enterocytes, which recognizes and respond to microbial components via pattern recognition receptors, several other cell subsets with immunological properties are found in the epithelial layer, including goblet cells (mucus producers), intraepithelial lymphocytes, DCs, macrophages, Paneth cells, Microfold (M) cells (transport of luminal particles), enteroendocrine cells (hormone producers) and tuft cells (initiation of type 2 immunity) [33, 106]. The crosstalk between this immunological compartment and the MALT is crucial for the full activation of gut immunity.

In the intestine, the MALT is named as gut-associated lymphoid tissue (GALT) and comprises subepithelial lymphoid aggregates, such as Peyer's patches (PPs) and isolated lymphoid follicles (ILFs). DCs, T and B cells in the GALT, immediately beneath the follicle-associated epithelium, are exposed to luminal antigens transported by M cells [76]. Specialized subsets of CD103-expressing DCs in the lamina propria and PPs produce high levels of IL-10, TGF-β and retinoic acid, therefore

promoting the differentiation of regulatory T cells and the expression of gut-homing molecules CCR9 and α4β7 on activated lymphocytes [48, 64]. The regulatory T cells induced in the gut mucosa are essential for the tolerance towards environmental antigens and commensal microbiota. In contrast, lower concentrations of TGF-β, and IL-6 in the mesenteric milieu induce the differentiation of Th17 cells [114]. Both, Th17 and regulatory T cells are the canonical responses in the gut mucosa shaped by the resident microbiota and its metabolites, such as short chain fatty acids [49, 50]. In contrast to the immunomodulatory effects of the regulatory T cells, the IL-17A produced by Th17 cells acts on intestinal epithelium to promote barrier function. In addition, together with IL-17F, IL-21, IL-22, TNF-α, IL-17 is critical in controlling extracellular infections. Th17 and regulatory T cells can also be converted into follicular T helper cells that support intestinal IgA production [40, 103]. Recently, adipose tissue depots adjacent to the intestine, particularly the mesentery, have been described as an important reservoir for memory T cells activated towards intestinal antigens [37]. As discussed later in this chapter, the adipose tissue provides a unique environment for the long-term survival of memory T cells that undergo metabolic reprogramming. In this context, the interplay between the metabolites, microbiota, epithelial and immune cells is critical to shaping the tissue-specific responses during homeostasis and in the face of infectious challenges. The disruption of this fine-tuned balance directly impacts the canonical pathways of the gut immune response and defines the outcome of infection and inflammatory diseases.

2 Consequences of Infection for the Tissue Immune Homeostasis

As described previously in this chapter, the barrier-associated immune system is endowed with tissue-specific mechanisms to ensure immunity, tolerance towards innocuous challenges and protection against tissue injury. However, defined pathogens can escape the firewall mechanisms and disrupt the tissue homeostasis, initiating infection and inflammatory process. Success in combating infectious disease consists of activating an innate immune response to prevent the growth of pathogens, induce an inflammatory response and promote the generation of adaptive immunity. However, pathogens are able to modulate the immune response to ensure their survival, which usually promotes a decrease in the tolerogenic response and increases the pro-inflammatory profile in the barrier tissues. For instance, years ago, several studies demonstrated that the protozoa *Leishmania* spp. has developed mechanisms to subvert macrophage killing through the modification of host cytokine expression such as IL-1 and TNF-α, downmodulation of major histocompatibility complex (MHC) class I and II by IFN-γ and reduction of ROS production [15, 23, 81].

Not only protozoan but also several other pathogens are capable of subverting macrophage or neutrophil microbicidal function, including bacteria and fungi. In the urogenital tract, for instance, the uropathogenic *Escherichia coli* has been shown

to suppress NF-κB activation in urothelial cells, decreasing cytokine secretion and increasing apoptosis [10, 43]. *Salmonella typhimurium* infection by the oral route impairs the adaptive immune response by promoting MHC class II ubiquitination in infected DCs, decreasing antigen presentation to CD4 T cells, and favoring bacterial survival [56]. Fungal species such as *Candida* spp. are part of the microbiome, but are considered pathobionts. Of these species, *C. albicans* is the most relevant, promoting oral thrush and vulvovaginal candidiasis. Neutrophils and macrophages have a relevant role in controlling mucosal fungal infections. However, studies have shown that *C. albicans* could escape neutrophils (NETose) and extracellular neutrophil traps (NETs) that release DNAse into the extracellular medium [112] and form biofilms [52], which renders fungal cells less accessible to immune cells. *C. albicans* also influence polarization of macrophages from a classic phenotype toward an alternative phenotype, thereby reducing antimicrobial response and promoting fungal survival. These are just a few examples about how pathogens evade specific cellular pathways of immunity. However, infections can compromise not only the functioning of defined immune cell subsets but also may interfere in the entire firewall mechanism.

Indeed, it has been described that a single gastrointestinal episode of infection with the bacteria *Yersinia pseudotuberculosis* can lead to chronic inflammation in several tissues, particularly the mesentery, long-term after the clearance of the pathogen. The infection impairs the immune dialog in the gut by changing the function of mesenteric lymphatic vessels. As a result, migratory DCs in the gut are deviated from the gut-lymph node migratory route, which permanently compromises the canonical regulatory responses in the mucosa, impacting mucosal immunity and promoting chronic inflammation [30]. Another example of immunological scar driven by infection, is the oral infection with *Toxoplasma gondi.* Although the systemic infection with *T. gondii* triggers a transient activation of IFN-γ- and TNF-producing Th1 cells, essential to infection control, there is also a persistent thymic atrophy and decrease in the naïve CD4 T lymphocyte pool. The result of the loss of thymic architecture supports chronic *T. gondii* infection and compromises resistance to other pathogens [54]. In the context of immune modulation, in 2019, two complementary studies demonstrated that measles virus (MV) infection, efficiently transmitted by aerosol or respiratory droplets, diminishes preexisting antibodies to previously encountered pathogens, a phenomenon called "immunological amnesia" [62], due to depletion of expanded B cell memory clones, and inability to restore the naïve B cell pool [77], and consequently, increasing vulnerability to future infections.

Therefore, despite the sophisticated mechanisms of barrier immunity, pathogens evolved to subvert the immune system and establish infection. For a very long time, the studies on host–pathogen interaction at barrier tissues had almost exclusively focused on the immunological aspects of the responses. However, only recently, the involvement of host and pathogen metabolic reprogramming began to be appreciated for understanding the outcome of infection. Indeed, the immune system adapts to the infectious environment to obtain nutrients used in cellular functions. The presence of pathogens at barrier sites, such as the lung and intestine, interfere with immune cell metabolism and pathogens modulate host cell metabolism to their advantage. In the next sections, will be discussed how cells of the innate and adaptive immune systems

induce a metabolic reprogramming necessary for antimicrobial molecules production and cell activation, proliferation and differentiation. Such changes also allow tissue repair and the generation of memory cells against future pathogen encounters.

3 Effects of Infection in the Immune Cell Metabolism

The cellular metabolic state is directly regulated by the surrounding microenvironment. Metabolic changes occur as an alternative to maintain homeostasis and control infections. However, metabolism also reflects the cell activation profile and, consequently, supports the immune response against pathogens. Metabolic processes are divided into anabolic processes, gathering macromolecules, and catabolic processes with the disruption of macromolecules. The catabolic process provides carbon and nitrogen sources, mainly through the use of glucose and glutamine, for the biosynthesis of nucleotides, amino acids, and lipids. In this way, the immune cell supports its activation and proliferation process. Each immune cell type has its own metabolic choice depending on its energy requirement and nutrient demand. For this purpose, cells have specific nutrient sensors and transcription of metabolic enzymes that assist in targeting the metabolism [58]. In the absence of glucose and glutamine, immune cells use fatty acids and amino acids as new sources of nutrients. Cellular energy is obtained through a catabolic process of high (oxidative phosphorylation) and low (glycolysis) efficiency. Quiescent cells use glucose degradation to generate pyruvate that supplies Tricarboxylic acid cycle (TCA) and oxidative phosphorylation. Unlike resting cells, classically activated M1 macrophages, DCs and effector T cells induce a metabolic reprogramming to support cellular biosynthetic processes termed "Warburg effect", often driven by aerobic glycolysis [25] (Fig. 2). Therefore, the uptake of nutrients is crucial to supply the cell demand for energy and favor metabolic shift. Likewise, pathogens use the same nutrients for their maintenance, which drives the competition for the available glucose [102].

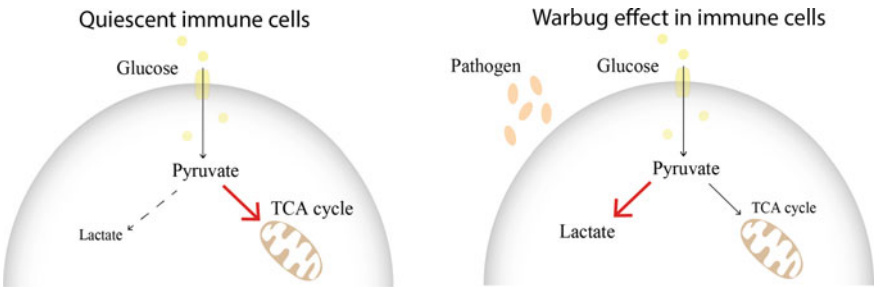


Fig. 2 Metabolic reprogramming in immune cells during infection. Quiescent cells maintain the glucose influx to supply the TCA cycle. Upon infection, innate immune cells increase glycolytic flux that culminate in lactate production even with oxygen availability (Warburg effect) and decrease in TCA cycle. TCA - Tricarboxylic acid cycle

Microbial compounds such as lipopolysaccharide (LPS) are recognized by Toll-like receptors (TLRs) in immune cells. This engagement induces a metabolic reprogramming in the innate immune cells to supply their current needs. In addition to microbial products, the inflammatory milieu drives cell differentiation and metabolism. Macrophage differentiation occurs according to the production of cytokines at the inflamed site into cell subsets that diverge mainly in their metabolic state. The cytokine IL-4 increases mitochondrial respiration and oxidation of fatty acids that culminate in the production of Arginase-1 (Arg-1) in macrophages named M2. However, IFN-γ increases the glycolytic pathway and the production of antimicrobial peptides such as the superoxide burst (ROS) and reactive nitrogen intermediates (NO)[101], characterizing a population of pro-inflammatory macrophages also known as M1. Although the polarization of macrophages is important for the outcome of the infection, the front line in combating pathogens at mucosal sites are tissue-resident macrophages (TRMs) that are specialized immune sentinels. In this way, TRMs change their metabolic reprogramming according to the environment. Upon IL4 stimulation, alveolar and peritoneal macrophages can undergo STAT6 phosphorylation, but only peritoneal macrophages upregulate Arg-1 [99]. Therefore, the escape mechanisms used by pathogens in TRMs may differ depending on the entry barrier. Indeed, the TRMs metabolism is mostly unknown due to its heterogeneity in barrier tissues, making this a crucial issue that must be addressed in future studies on cell metabolic reprogramming.

Like macrophages, the activation of DCs, mast cells and neutrophils increases the glycolytic pathway and fatty acid synthesis. In addition to switching to the glycolytic pathway, metabolic reprogramming of innate immunity cells triggers several metabolic changes after the pathogen recognition. The infected cell activates the phosphoinositide-3 kinase/Akt (PI3K / Akt) pathway to induce a signaling cascade that supports metabolic reprogramming. PI3K promotes phosphatidylinositol-3,4,5-trisphosphate (PIP3) which conscripts Akt to be activated by Phosphoinositide-dependent kinase 1 (PDK1) [31]. Phosphorylated Akt prompts the glucose transporter 1 (GLUT1), hexokinase 2 (HK2), and phosphofructokinase 1 (PFK1) expression. Next, Akt induces ATP-dependent citrate lyase (ACL), which converts citrate in the cytosol into oxaloacetate (OAA) and Acetyl-CoA. The metabolic switch to the glycolytic pathway reduces pyruvate entry into mitochondrial TCA and increases lactate production. In this context, the TCA pathway is supplied by the increase in glutaminolysis, providing α-ketoglutarate (α-KG) and, consequently, precursors of amino acids. Thus, the citrate released into the cytosol is converted to OAA and acetyl-CoA for the synthesis of lipids and fatty acids. The citrate is also a precursor for the itaconate acid [61], which has antimicrobial functions against intracellular bacteria from lung barriers such as Mycobacterium *tuberculosis* and *Legionella pneumophila*; or intestinal barriers such as *Salmonella enterica* [59]. Still, citrate is also used to develop reactive oxygen species (ROS), nitric oxide (NO) and prostaglandins. The accumulation of succinate induced by high levels of pyruvate kinase M2 (PKM2) after LPS recognition, stabilizes hypoxia-inducible factor 1 alpha (HIF1a) allowing translocation to the nucleus and inducing IL1 transcription [100]. Oxidized succinate

induces mROS production and also assists in HIF1a-mediated IL1b mRNA expression. In contrast, glucose 6-phosphate triggers the Pentose Phosphate Pathway (PPP), which culminates in reducing equivalent NADPH and NADH, that will be used for the generation of ROS and NO (Fig. 3).

The recognition of molecular patterns is crucial for inducing metabolic reprogramming during infection. Notably, it has been observed that defined stimuli, such as β-glucan and Bacillus Calmette-Guérin (BCG), recognized by innate immune cells induce long-term functional reprogramming, which is referred to as "trained immunity", which is an innate version for immunological memory [77, 85]. The changes occur through epigenetic reprogramming due to histones acetylation and methylation in monocyte, macrophage or NK cells that culminate in an increase in cytokine production and metabolic shift [85]. Therefore, trained immunity increases responsiveness to second infections for weeks and the ability to eliminate the pathogen.

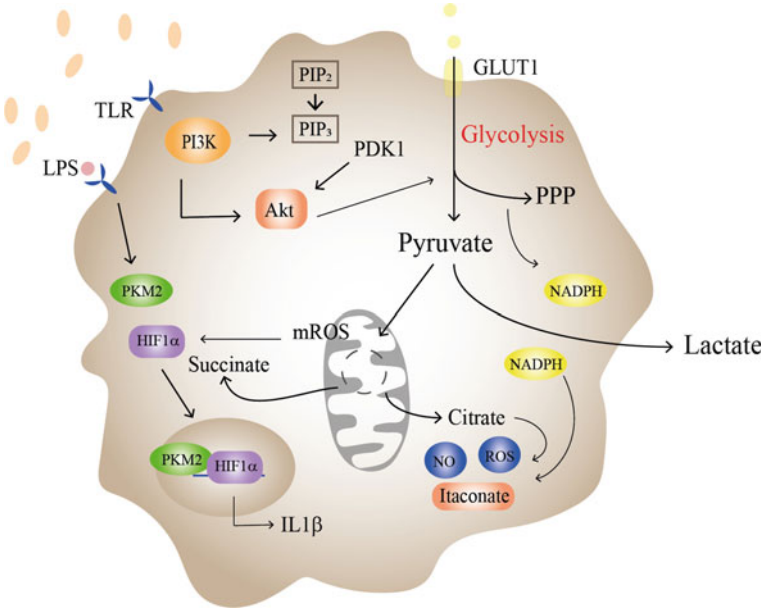


Fig. 3 Metabolic reprogramming in innate immune cells during infection. Microbial compounds increase glycolysis through Akt activation and induce PKM2 dimers and tetramers. PKM2 dimers translocate to the nucleus and contribute to IL1β expression. Accumulated succinate stabilizes HIF1α to drive IL1β expression and citrate promotes NO, ROS and itaconate production

4 Metabolic Reprogramming of T Cells to Protect Against Infections

Similar to innate immune cells, activated T cells also induce metabolic reprogramming to support cell biosynthesis. After activation by antigen and costimulatory molecules, T cells increase glycolysis and oxidative phosphorylation (OXPHOS) to supply the energy demand. High expression of glucose transporters (Glut1) supports glucose uptake to maintain the functional glycolytic pathway. In addition, Glut1 expression increases T cell proliferation, cytokine production and cell survival. In naïve T cells, Glut1 expression is low and is increased after activation of TCR and CD28-mediated Akt-dependent and independent pathways [46]. T-cell glycolytic metabolism is restrained by the mammalian target of rapamycin (mTOR) complex 1 (mTORC1) activity and transcription factors targeting metabolic reprogramming, such as HIF1 and c-Myc [91, 107]. In addition, under low nutrient availability, T cells adapt to low glucose levels, increasing glutaminolysis through glutamine uptake [11]. Glutamine uptake is controlled by the ERK / MAPK pathway during T lymphocyte activation [16].

Effector T cells maintain the glycolytic pathway after differentiation, while Foxp3 + regulatory T cells decrease glucose consumption and prefer to use derived fatty acids to support oxidative metabolism [34]. Besides its own metabolism, the maintenance and generation of regulatory cells in barrier sites depend on homeostatic environmental control. The production of TGFβ, prostaglandin E2 (PGE2) and IL-10 by the mucosal epithelium, down regulates the immune response and induces regulatory T cells [4, 53, 74]. As discussed previously, the presence of regulatory T cells is essential for mucosal tolerance, and the mucosal surface portrays a complex system that must work to contain harmful pathogens, but at the same time, it must inhibit the immune response against commensal microorganisms, proper antigens and nutrients. Thereby, mucosal components provide significant metabolic changes, mainly targeting tissue hypoxia for environmental control or supporting metabolic shift in T cells. Intraepithelial cells detect the pathogen and promote metabolic changes in TCRγδ intestinal intraepithelial lymphocytes, increasing the glycolytic pathway to sustain the maintenance of the intestinal epithelial barrier against enteric pathogens [41]. However, T-cell subpopulations have their own energy demand and metabolic reprogramming to perform their immune function.

Contrary to Foxp3 + regulatory T cells, type 1 regulatory T cells (Tr1) maintain the glycolytic pathway as effector T cells. Meanwhile, memory T cells predominantly use fatty acid oxidation to support oxidative phosphorylation (OxPhos), and glucose is used to generate mitochondrial citrate (Fig. 4). However, in an infectious context, the metabolic reprogramming of T cells depends on the environmental conditions and available nutrients that result in efficient activation, regulation and memory generation. Chronic infections such as *Mycobacterium tuberculosis*, mitochondrial dysfunction in CD8 T cells result in greater dependence on glycolysis and show bioenergetic deficiencies [84]. However, for memory T cells generation,

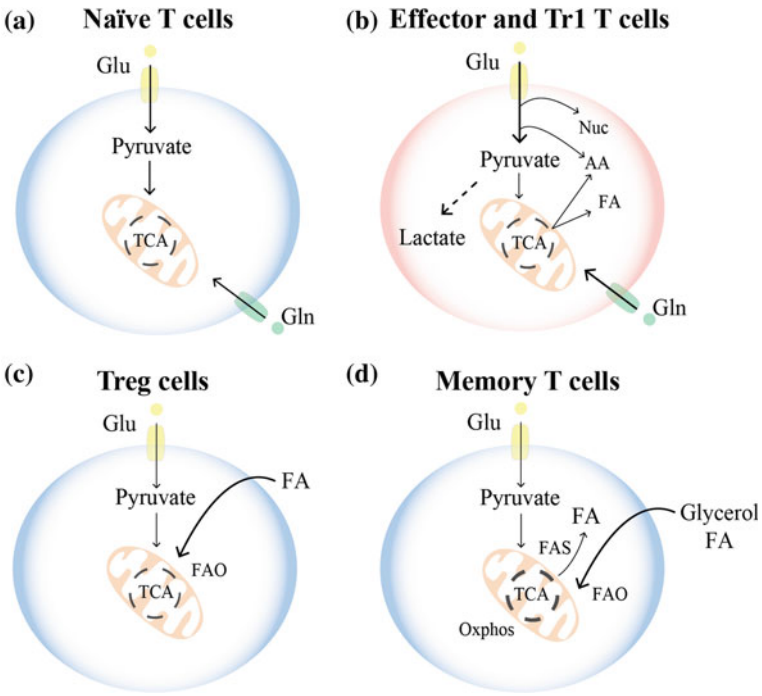


Fig. 4 Metabolic pathway in T cell subsets. A. Naive T cells use glucose and glutamine to maintain the oxphos pathway. B. Effector and Tr1 cells increase glycolysis and produce lactate. Intermediate components support the synthesis of nucleotides (Nuc), amino acids (AA) and fatty acids (FA). C. Foxp3^{+} T regs use extracellular FA to support oxphos by fatty acid oxidation (FAO). D. Memory T cells use glucose to increase TCA intermediate components to support fatty acid synthesis (FAS). Glycerol and FA are used to fuel the oxphos pathway. Blue cells show oxidative metabolism and red cells show glycolytic metabolism

the glycolytic pathway is reduced, and the AMP-activated protein kinase (AMPK) activity and OxPhos are increased for homeostatic maintenance.

5 Modulation of Immune Cell Metabolism by Pathogens

As discussed previously, immune cells that compose an active barrier at sites of high exposure to pathogens control infections and maintain the body's homeostasis. For this, immune cells need to change the metabolism and provide energy even with the drop of nutrients in the environment. In contrast, pathogens have mechanisms that act as an opposite force for their survival within the host. Intracellular pathogens, such as viruses and some bacteria, are able to reprogram host cell metabolism to increase the demand for nutrients and allow their replication. Elseways, extracellular pathogens modulate the immune system to improve the production of essential metabolites for

their survival. Herein, we will discuss how pathogens modulate the metabolism of immune cells and determine their permanence during infection.

The limited resources of cellular energy during the pathogen-host interaction lead to a race against time in obtaining nutrients for the cellular machinery of both. In addition, metabolic changes in the host cell may favor the pathogen's permanence due to the evasion of the immune system. In this way, metabolic changes act as key mechanisms for the survival of some species, mainly by increasing glucose and lactate in the host cell. Some protozoa use the host's glucose as a primary carbon source, such as *Trypanosoma brucei* and *Plasmodium falciparum* [75, 89]. The extracellular pathogenic bacterium group A *Streptococcus* (GAS) causes an endoplasmic reticulum stress to host cells. This stress increases asparagine (ASN) synthetase expression that promotes GAS proliferation, while proliferation-linked genes are downregulated in the absence of ASN [6]. Another extracellular pathogen, the *Citrobacter rodentium*, modulates innate immunity and microbiota composition, impairing cellular bioenergetics [65].

In general, the modulation of host metabolism guarantees nutrients and evasion of the immune system to increase the pathogen survival. Thereby, the metabolic shift in the host cell promotes the intracellular survival *of Mycobacterium tuberculosis* [9], *Legionella pneumophila* [29], *Brucella abortus* [20] or *Chlamydia trachomatis* [83]. Cancer cells, known to induce the Warburg effect, are more susceptible to *Listeria monocytogenes* replication, suggesting that metabolic reprogramming is a favorable mechanism for the survival of this bacterium [35]. Among the many infections that interfere with the host metabolism, a group of pathogenic bacteria, known as Intracellular Bacterial Pathogens (IBPs), survive and proliferate inside vacuoles or in the cytosol for long periods. Because IBPs are heterotrophic, i.e., metabolism depends on carbon sources for energy,they use both the host carbon and nitrogen. An interesting fact is a possible association between intracellular carbon metabolism and virulence gene expression. It is known that specific regulators can control these genes that sense metabolites generated by the metabolism of carbon and nitrogen [27]. As a primary carbon source, IBPs use glucose from immune cells for their metabolic activities [13]. However, defined bacteria need a "biopartite metabolism" because they have more difficulty in using only glucose. These IBPs use several sources of carbon from the host to generate energy and stay within the host cells. Among them, it is possible to highlight TCA intermediaries such as succinate and malate, amino acids, fatty acids and also products derived from glucose such as glycerol (-3P), pyruvate and lactate [28]. Due to the evolutionary adaptation of the pathogen, some components in the metabolic reprogramming of the host cell, are used as an escape and survival mechanism. Alternatively, pathogen virulence factors interact with central regulators of host metabolism (Fig. 5). The activation of PI3K/Akt pathway is downmodulated by the Src homology 2 (SH2) domain-containing inositol-5′-phosphatase (SHIP) protein that promotes *Francisella tularensis* to escape into the cytosol [3, 19]. Still, the motility and phagocytosis of the host cell are controlled by molecular mechanisms developed by IBPs subverting the metabolism of phosphoinositides [78]. In experimental model, the infection with *Yersinia enterocolitica* led to HIF-1 in Peyer's patches [39]. Furthermore, Salmonella requires the fatty acid regulator

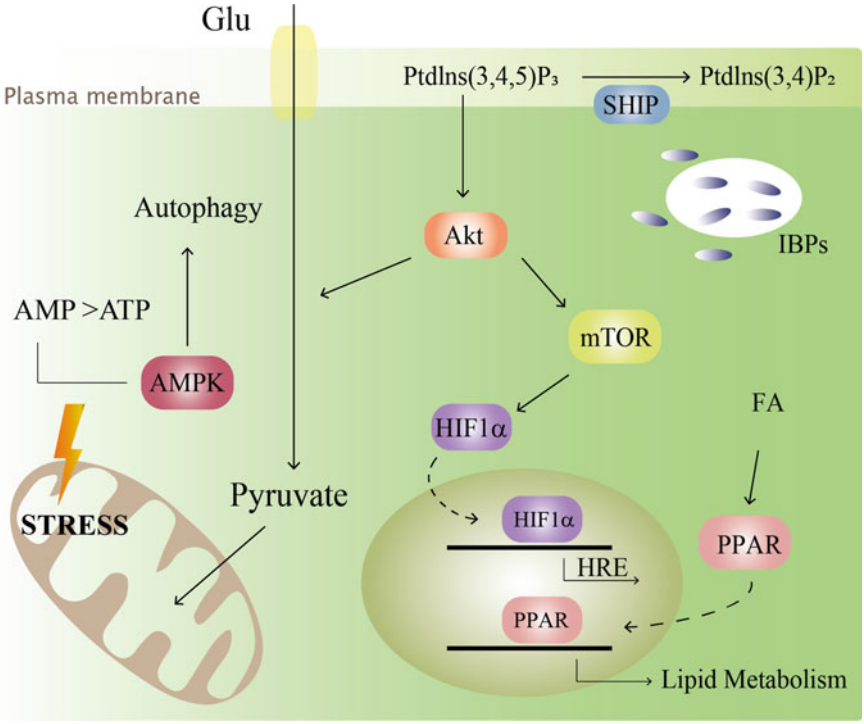


Fig. 5 Intracellular bacterial pathogens (IBPs) modulate central metabolic regulators in the host cells. Bacterial components activate or alter metabolic pathways to evade the immune system and maintain its survival. The main metabolic mediators affected by IBPs are the Pi3K/Akt/mTOR pathway, HIF1α, fatty acid regulators and AMPK

PPARδ to establish a metabolic environment that favors a long-term permanence. Under cellular stress, defined metabolic strategies to obtain nutrients also promote the elimination of intracellular pathogens. These cells reach high levels of AMP/ATP by reducing the production of ATP, which in turn induces the AMP-activated protein kinase (AMPK) activation. Therefore, AMPK regulates the autophagic process, efficient autophagosome maturation [47]. Thus, autophagy is used as a self-degrading process to obtain energy, and hence intracellular pathogens can be eliminated as well. However, pathogens have machinery capable of modulating autophagy in the host, either by changing the maturation of the phagosome such as *Mycobacteria* [93], *Legionella* [90], *Brucella* [97] and *Salmonella* [108], or escaping from those vacuoles into the cytoplasm as in *Shigella* [72] and *Listeria monocytogene*s infection [63]. Therefore, pathogens evade host defense mechanisms not only by interfering in the microbicide function of immune cells, but also, in their metabolic pathways.

In general, the viruses perform both lytic cycles, with high replication and viral load, as well as latent and persistent within the cell. Therefore, these conditions have different metabolic needs of the host cell [21]. During viral replication, the

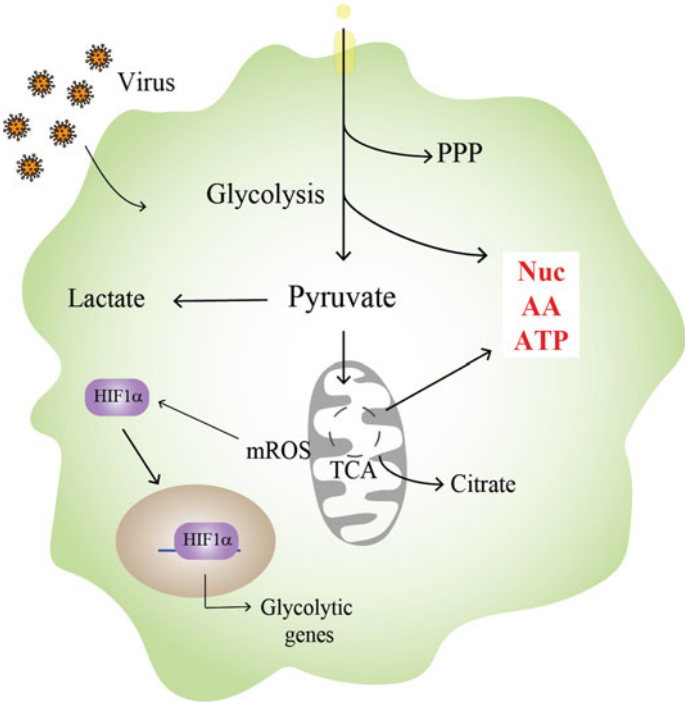


Fig. 6 Virus infection modulates host cell metabolism to support its replication. Viruses promote metabolic changes in host cells to increase the production of Nucleotides (Nuc), amino acids (AA) and ATP that support viral replication. Entry into the glycolytic pathway is a critical mechanism to supply the demand for viral components

virus depends on high demand for nucleotides, amino acids and ATP (Fig. 6). Therefore, changes in glycolytic pathways, fatty acid synthesis (FAS), glutaminolysis and Pentose Phosphate Pathway (PPP) are observed. The targeting of glucose to PPP supports the production of nucleotides. In addition, pyruvate can be converted to lactate or enter the TCA pathway. Citrate can be transported to the cytoplasm via the FAS pathway. On the other side, glutamine enters the infected cell and supports the TCA cycle [87]. Therefore, the catabolism of suitable carbon sources in infected cells is crucial for viral replication, enabling the synthesis of nucleic acids and the viral envelope. For instance, the infection of monocytes with acute respiratory syndrome coronavirus 2 (SARS-CoV-2) triggers mitochondrial ROS production, capable of HIF-1α stabilization, which promotes the expression of glycolytic genes and IL-1β, responsible to monocytes metabolic reprogramming to aerobic glycolysis (Warburg effect) and T cell dysfunction, respectively. These data may explain how diabetic individuals with uncontrolled glucose levels are more susceptible to develop the severe form of Coronavirus disease 2019 (COVID-19), the pandemic that affected the world's population in 2020 [18].

Another example of metabolic reprogramming in the context of viral infection is the HIV. The susceptibility of different subtypes of CD4 T cells to HIV-1 infection is associated with high cellular metabolic activity. In fact, independently of their activation phenotype, CD4 T cells with high oxidative phosphorylation and glycolysis are more infected by HIV as compared to CD4 T cells harboring other metabolic profiles [105]. As exemplified above, different viruses are capable of infecting specific barrier tissues, such as COVID-19 respiratory tract infection and HIV-1 genital tract infection. However, regardless of the virus or the infected barrier site, one of the characteristics observed is the ability that these viruses to interfere within the metabolism of immune cells in favor of their survival and replication. Most of the studies regarding immune cell metabolism were performed in vitro, and now this knowledge needs to be integrated into the context of tissue-specific immune responses. Therefore, we need to better understand how the cellular metabolic reprogramming imposed by different types of pathogens can interfere, especially in the long-term, in the barrier immune response and its function, given the relevance of the balance between expansion and contraction of effector immune response in these tissues and their role as pathogen entrance sites.

6 Therapy Targeting Cell Metabolism to Control Infections

Considering that intracellular as well as extracellular pathogens can promote the metabolic reprogramming of the host immune cells, either through the modification of the cellular immune response profile or even the bioavailability of nutrients, it is plausible to suggest that therapies targeting metabolic pathways may be important to better target the immune response to fight the infectious agent and return homeostasis. In contrast to the pathogen-targeted traditional antimicrobial therapy, these therapeutic approaches would target the host metabolism.

For instance, the first line of oral drugs for controlling glucose metabolism is metformin, that activates AMP kinase, therefore lowering blood glucose concentrations by decreasing hepatic gluconeogenesis and improving glucose cell uptake. Because of this, metformin is commonly used to treat diabetes. One of the anti-inflammatory effects associated with metformin seen in LPS-activated macrophages is the inhibition ROS generation through reverse electron transport at Complex I and inhibition of IL-1β production [51]. Another one is to induce the inhibition of Complex I, which consequently reduces intracellular ATP production and activation of AMP-activated protein kinase (AMPK) [98, 113]. In contrast, in an AMPK-independent manner, metformin has been shown to inhibit the mammalian target of rapamycin complex I (mTORCI) in T cells and prevents the expression of the transcription factors c-Myc and Hif-1α [111]. Although metformin treatment is associated with a decreased risk of developing sepsis in humans [92],in experimental model it has been suggested that metformin may increase *C. albicans* infections by reducing

macrophages responses to this opportunistic fungal infection [104]. Another anti-inflammatory drug is dimethyl fumarate, which promotes the expression of antioxidant genes by stabilizing the expression of the transcription factor Nuclear Factor (erythroid-derived 2) like 2 (Nrf2) [24] and is usually prescribed for treating multiple sclerosis (MS) [12] and psoriasis [14]. The Nrf2 regulates the pentose phosphate route in macrophages, reducing inflammatory macrophages response and induces lipid metabolism alterations in MS patients. In addition, fungal β-glucan administration is demonstrated to induce trained immunity characterized by increased glycolysis and partial reversion of LPS-induced immunoparalysis in monocytes isolated from human blood [71]. Moreover, oral glucan administration also demonstrated increased survival in experimental models challenged with *S. aureus*, as well as *C. albicans,* demonstrating that trained immunity is not a pathogen-specific response [82]. The world has been witnessing a constant emergence of new pathogens against which there may be no effective antimicrobial drugs. Therefore, the manipulation of metabolic cell reprogramming may represent a new promising approach for treating infectious diseases.

References

1. Abraham SN, ST John A L (2010) Mast cell-orchestrated immunity to pathogens. Nat Rev Immunol 10:440–452
2. Agace WW, Hedges SR, Ceska M, Svanborg C (1993) Interleukin-8 and the neutrophil response to mucosal gram-negative infection. J Clin Invest 92:780–785
3. Asare R, Kwaik YA (2010) Exploitation of host cell biology and evasion of immunity by francisella tularensis. Front Microbiol 1:145
4. Baratelli F, Lin Y, Zhu L, Yang SC, Heuze-Vourc'h N, Zeng G, Reckamp K, Dohadwala M, Sharma S, Dubinett SM (2005) Prostaglandin E2 induces FOXP3 gene expression and T regulatory cell function in human CD4+ T cells. J Immunol 175:1483–1490
5. Barlow JL, McKenzie ANJ (2019) Innate Lymphoid Cells of the Lung. Annu Rev Physiol 81:429–452
6. Baruch M, Belotserkovsky I, Hertzog BB, Ravins M, Dov E, McIver KS, le Breton YS, Zhou Y, Cheng CY, Hanski E (2014) An extracellular bacterial pathogen modulates host metabolism to regulate its own sensing and proliferation. Cell 156:97–108
7. Belkaid Y, Hand TW (2014) Role of the microbiota in immunity and inflammation. Cell 157:121–141
8. Belkaid Y, Harrison OJ (2017) Homeostatic Immunity and the Microbiota. Immunity 46:562–576
9. Billig S, Schneefeld M, Huber C, Grassl GA, Eisenreich W, Bange FC (2017) Lactate oxidation facilitates growth of Mycobacterium tuberculosis in human macrophages. Sci Rep 7:6484
10. Billips BK, Schaeffer AJ, Klumpp DJ (2008) Molecular basis of uropathogenic Escherichia coli evasion of the innate immune response in the bladder. Infect Immun 76:3891–3900
11. Blagih J, Coulombe F, Vincent EE, Dupuy F, Galicia-Vazquez G, Yurchenko E, Raissi TC, van der Windt GJ, Viollet B, Pearce EL, Pelletier J, Piccirillo CA, Krawczyk CM, Divangahi M, Jones RG (2015) The energy sensor AMPK regulates T cell metabolic adaptation and effector responses in vivo. Immunity 42:41–54
12. Bomprezzi R (2015) Dimethyl fumarate in the treatment of relapsing-remitting multiple sclerosis: an overview. Ther Adv Neurol Disord 8:20–30

13. Bowden SD, Rowley G, Hinton JC, Thompson A (2009) Glucose and glycolysis are required for the successful infection of macrophages and mice by Salmonella enterica serovar typhimurium. Infect Immun 77:3117–3126
14. Bruck J, Dringen R, Amasuno A, Pau-Charles I, Ghoreschi K (2018) A review of the mechanisms of action of dimethylfumarate in the treatment of psoriasis. Exp Dermatol 27:611–624
15. Buchmuller-Rouiller Y, Mauel J (1987) Impairment of the oxidative metabolism of mouse peritoneal macrophages by intracellular Leishmania spp. Infect Immun 55:587–593
16. Carr EL, Kelman A, Wu GS, Gopaul R, Senkevitch E, Aghvanyan A, Turay AM, Frauwirth KA (2010) Glutamine uptake and metabolism are coordinately regulated by ERK/MAPK during T lymphocyte activation. J Immunol 185:1037–1044
17. Chan, C. Y., ST John AL, Abraham SN (2013) Mast cell interleukin-10 drives localized tolerance in chronic bladder infection. Immunity 38:349–359
18. Codo AC, Davanzo GG, de Brito Monteiro L, de Souza GF, Muraro SP, Virgilio-da-Silva JV, Prodonoff JS, Carregari VC, de Biagi Junior CA, Crunfli F, Restrepo JL (2020) Elevated Glucose Levels Favor SARS-CoV-2 Infection and Monocyte Response through a HIF-1alpha/Glycolysis-Dependent Axis. Cell Metab 32:498–499
19. Cremer TJ, Butchar JP, Tridandapani S (2011) Francisella Subverts Innate Immune Signaling: Focus On PI3K/Akt. Front Microbiol 5:13
20. Czyż DM, Willett JW, Crosson S (2017) Brucella abortus Induces a Warburg Shift in Host Metabolism That Is Linked to Enhanced Intracellular Survival of the Pathogen. J Bacteriol, 199
21. Delgado T, Sanchez EL, Camarda R, Lagunoff M. (2012)Global metabolic profiling of infection by an oncogenic virus: KSHV induces and requires lipogenesis for survival of latent infection. PLoS Pathog, 8 e1002866.
22. Delima AJ, van Dyke TE (2003) Origin and function of the cellular components in gingival crevice fluid. Periodontol 2000(31):55–76
23. Descoteaux A, Matlashewski G (1989) c-fos and tumor necrosis factor gene expression in Leishmania donovani-infected macrophages. Mol Cell Biol 9:5223–5227
24. Deshmukh P, Unni S, Krishnappa G, Padmanabhan B (2017) The Keap1-Nrf2 pathway: promising therapeutic target to counteract ROS-mediated damage in cancers and neurodegenerative diseases. Biophys Rev 9:41–56
25. Donnelly RP, Finlay DK (2015) Glucose, glycolysis and lymphocyte responses. Mol Immunol 68:513–519
26. Dutzan N, Abusleme L, Bridgeman H, Greenwell-Wild T, Zangerle-Murray T, Fife ME, Bouladoux N, Linley H, Brenchley L, Wemyss K, Calderon G, Hong BY, Break TJ, Bowdish DM, Lionakis MS, Jones SA, Trinchieri G, Diaz PI, Belkaid Y, Konkel JE, Moutsopoulos NM (2017) On-going Mechanical Damage from Mastication Drives Homeostatic Th17 Cell Responses at the Oral Barrier. Immunity 46:133–147
27. Eisenreich W, Heesemann J, Rudel T, Goebel W (2013) Metabolic host responses to infection by intracellular bacterial pathogens. Front Cell Infect Microbiol 3:24
28. Eisenreich W, Rudel T, Heesemann J, Goebel W (2017) To Eat and to Be Eaten: Mutual Metabolic Adaptations of Immune Cells and Intracellular Bacterial Pathogens upon Infection. Front Cell Infect Microbiol 7:316
29. ESCOLL, P., SONG, O. R., VIANA, F., STEINER, B., LAGACHE, T., OLIVO-MARIN, J. C., IMPENS, F., BRODIN, P., HILBI, H. & BUCHRIESER, C. 2017. Legionella pneumophila Modulates Mitochondrial Dynamics to Trigger Metabolic Repurposing of Infected Macrophages. *Cell Host Microbe,* 22**,** 302–316 e7.
30. Fonseca DM, Hand TW, Han SJ, Gerner MY, GLATMAN ZARETSKY, A., BYRD, A. L., HARRISON, O. J., ORTIZ, A. M., QUINONES, M., TRINCHIERI, G., BRENCHLEY, J. M., BRODSKY, I. E., GERMAIN, R. N., RANDOLPH, G. J. & BELKAID, Y. (2015) Microbiota-Dependent Sequelae of Acute Infection Compromise Tissue-Specific Immunity. Cell 163:354–366
31. FRANKE, T. F. 2008. Intracellular signaling by Akt: bound to be specific. *Sci Signal,* 1**,** pe29.

32. Gallo RL, Hooper LV (2012) Epithelial antimicrobial defence of the skin and intestine. Nat Rev Immunol 12:503–516
33. Gerbe F, Sidot E, Smyth DJ, Ohmoto M, Matsumoto I, Dardalhon V, Cesses P, Garnier L, Pouzolles M, Brulin B, Bruschi M, Harcus Y, Zimmermann VS, Taylor N, Maizels RM, Jay P (2016) Intestinal epithelial tuft cells initiate type 2 mucosal immunity to helminth parasites. Nature 529:226–230
34. Gerriets VA, Rathmell JC (2012) Metabolic pathways in T cell fate and function. Trends Immunol 33:168–173
35. GILLMAIER, N., GOTZ, A., SCHULZ, A., EISENREICH, W. & GOEBEL, W. 2012. Metabolic responses of primary and transformed cells to intracellular Listeria monocytogenes. *PLoS One,* 7**,** e52378.
36. Grist M, Chakraborty J (1994) Identification of a mucin layer in the urinary bladder. Urology 44:26–33
37. HAN, S. J., GLATMAN ZARETSKY, A., ANDRADE-OLIVEIRA, V., COLLINS, N., DZUTSEV, A., SHAIK, J., MORAIS DA FONSECA, D., HARRISON, O. J., TAMOUTOUNOUR, S., BYRD, A. L., SMELKINSON, M., BOULADOUX, N., BLISKA, J. B., BRENCHLEY, J. M., BRODSKY, I. E. & BELKAID, Y. 2017. White Adipose Tissue Is a Reservoir for Memory T Cells and Promotes Protective Memory Responses to Infection. *Immunity,* 47**,** 1154–1168 e6.
38. Haraoka M, Hang L, Frendeus B, Godaly G, Burdick M, Strieter R, Svanborg C (1999) Neutrophil recruitment and resistance to urinary tract infection. J Infect Dis 180:1220–1229
39. Hartmann H, Eltzschig HK, Wurz H, Hantke K, Rakin A, Yazdi AS, Matteoli G, Bohn E, Autenrieth IB, Karhausen J, Neumann D, Colgan SP, Kempf VA (2008) Hypoxia-independent activation of HIF-1 by enterobacteriaceae and their siderophores. Gastroenterology 134:756–767
40. Hirota K, Turner JE, Villa M, Duarte JH, Demengeot J, Steinmetz OM, Stockinger B (2013) Plasticity of Th17 cells in Peyer's patches is responsible for the induction of T cell-dependent IgA responses. Nat Immunol 14:372–379
41. HOYTEMA VAN KONIJNENBURG, D. P., REIS, B. S., PEDICORD, V. A., FARACHE, J., VICTORA, G. D. & MUCIDA, D. 2017. Intestinal Epithelial and Intraepithelial T Cell Crosstalk Mediates a Dynamic Response to Infection. *Cell,* 171**,** 783–794 e13.
42. Humphrey SP, Williamson RT (2001) A review of saliva: normal composition, flow, and function. J Prosthet Dent 85:162–169
43. Hunstad DA, Justice SS, Hung CS, Lauer SR, Hultgren SJ (2005) Suppression of bladder epithelial cytokine responses by uropathogenic Escherichia coli. Infect Immun 73:3999–4006
44. IWASAKI, A. (2007) Mucosal dendritic cells. Annu Rev Immunol 25:381–418
45. IWASAKI, A. (2010) Antiviral immune responses in the genital tract: clues for vaccines. Nat Rev Immunol 10:699–711
46. Jacobs SR, Herman CE, Maciver NJ, Wofford JA, Wieman HL, Hammen JJ, Rathmell JC (2008) Glucose uptake is limiting in T cell activation and requires CD28-mediated Akt-dependent and independent pathways. J Immunol 180:4476–4486
47. Jang M, Park R, Kim H, Namkoong S, Jo D, Huh YH, Jang IS, Lee JI, Park J (2018) AMPK contributes to autophagosome maturation and lysosomal fusion. Sci Rep 8:12637
48. Johansson-Lindbom B, Svensson M, Wurbel MA, Malissen B, Marquez G, Agace W (2003) Selective generation of gut tropic T cells in gut-associated lymphoid tissue (GALT): requirement for GALT dendritic cells and adjuvant. J Exp Med 198:963–969
49. Kayama H, Okumura R, Takeda K (2020) Interaction Between the Microbiota, Epithelia, and Immune Cells in the Intestine. Annu Rev Immunol 38:23–48
50. Kayama H, Takeda K (2020) Manipulation of epithelial integrity and mucosal immunity by host and microbiota-derived metabolites. Eur J Immunol 50:921–931
51. Kelly B, Tannahill GM, Murphy MP, O'Neill LA (2015) Metformin Inhibits the Production of Reactive Oxygen Species from NADH: Ubiquinone Oxidoreductase to Limit Induction of Interleukin-1beta (IL-1beta) and Boosts Interleukin-10 (IL-10) in Lipopolysaccharide (LPS)-activated Macrophages. J Biol Chem 290:20348–20359

52. KERNIEN, J. F., JOHNSON, C. J. & NETT, J. E. 2017. Conserved Inhibition of Neutrophil Extracellular Trap Release by Clinical Candida albicans Biofilms. *J Fungi (Basel),* 3.
53. Konkel JE, Chen W (2011) Balancing acts: the role of TGF-beta in the mucosal immune system. Trends Mol Med 17:668–676
54. Kugler DG, Flomerfelt FA, Costa DL, Laky K, Kamenyeva O, Mittelstadt PR, Gress RE, Rosshart SP, Rehermann B, Ashwell JD, Sher A, Jankovic D (2016) Systemic toxoplasma infection triggers a long-term defect in the generation and function of naive T lymphocytes. J Exp Med 213:3041–3056
55. LACERDA MARIANO, L. & INGERSOLL, M. A. (2020) The immune response to infection in the bladder. Nat Rev Urol 17:439–458
56. Lapaque N, Hutchinson JL, Jones DC, Meresse S, Holden DW, Trowsdale J, Kelly AP (2009) Salmonella regulates polyubiquitination and surface expression of MHC class II antigens. Proc Natl Acad Sci U S A 106:14052–14057
57. Lavin Y, Winter D, Blecher-Gonen R, David E, Keren-Shaul H, Merad M, Jung S, Amit I (2014) Tissue-resident macrophage enhancer landscapes are shaped by the local microenvironment. Cell 159:1312–1326
58. Loftus RM, Finlay DK (2016) Immunometabolism: Cellular Metabolism Turns Immune Regulator. J Biol Chem 291:1–10
59. Luan HH, Medzhitov R (2016) Food Fight: Role of Itaconate and Other Metabolites in Antimicrobial Defense. Cell Metab 24:379–387
60. Martinon F, Mayor A, Tschopp J (2009) The inflammasomes: guardians of the body. Annu Rev Immunol 27:229–265
61. Michelucci A, Cordes T, Ghelfi J, Pailot A, Reiling N, Goldmann O, Binz T, Wegner A, Tallam A, Rausell A, Buttini M, Linster CL, Medina E, Balling R, Hiller K (2013) Immune-responsive gene 1 protein links metabolism to immunity by catalyzing itaconic acid production. Proc Natl Acad Sci U S A 110:7820–7825
62. Mina MJ, Kula T, Leng Y, Li M, de Vries RD, Knip M, Siljander H, Rewers M, Choy DF, Wilson MS, Larman HB, Nelson AN, Griffin DE, de Swart RL, Elledge SJ (2019) Measles virus infection diminishes preexisting antibodies that offer protection from other pathogens. Science 366:599–606
63. Mitchell G, Cheng MI, Chen C, Nguyen BN, Whiteley AT, Kianian S, Cox JS, Green DR, McDonald KL, Portnoy DA (2018) Listeria monocytogenes triggers noncanonical autophagy upon phagocytosis, but avoids subsequent growth-restricting xenophagy. Proc Natl Acad Sci U S A 115:E210–E217
64. Mora JR, Iwata M, Eksteen B, Song SY, Junt T, Senman B, Otipoby KL, Yokota A, Takeuchi H, Ricciardi-Castagnoli P, Rajewsky K, Adams DH, von Andrian UH (2006) Generation of gut-homing IgA-secreting B cells by intestinal dendritic cells. Science 314:1157–1160
65. Mullineaux-Sanders C, Sanchez-Garrido J, Hopkins EGD, Shenoy AR, Barry R, Frankel G (2019) Citrobacter rodentium-host-microbiota interactions: immunity, bioenergetics and metabolism. Nat Rev Microbiol 17:701–715
66. Murakami M, Ohtake T, Dorschner RA, Schittek B, Garbe C, Gallo RL (2002) Cathelicidin anti-microbial peptide expression in sweat, an innate defense system for the skin. J Invest Dermatol 119:1090–1095
67. Nagamatsu K, Hannan TJ, Guest RL, Kostakioti M, Hadjifrangiskou M, Binkley J, Dodson K, Raivio TL, Hultgren SJ (2015) Dysregulation of Escherichia coli alpha-hemolysin expression alters the course of acute and persistent urinary tract infection. Proc Natl Acad Sci U S A 112:E871–E880
68. Naik S, Bouladoux N, Linehan JL, Han SJ, Harrison OJ, Wilhelm C, Conlan S, Himmelfarb S, Byrd AL, Deming C, Quinones M, Brenchley JM, Kong HH, Tussiwand R, Murphy KM, Merad M, Segre JA, Belkaid Y (2015) Commensal-dendritic-cell interaction specifies a unique protective skin immune signature. Nature 520:104–108
69. Naik S, Bouladoux N, Wilhelm C, Molloy MJ, Salcedo R, Kastenmuller W, Deming C, Quinones M, Koo L, Conlan S, Spencer S, Hall JA, Dzutsev A, Kong H, Campbell DJ, Trinchieri G, Segre JA, Belkaid Y (2012) Compartmentalized control of skin immunity by resident commensals. Science 337:1115–1119

70. NETEA, M. G., JOOSTEN, L. A., LATZ, E., MILLS, K. H., NATOLI, G., STUNNENBERG, H. G., O'NEILL, L. A. & XAVIER, R. J. 2016. Trained immunity: A program of innate immune memory in health and disease. *Science,* 352**,** aaf1098.
71. NOVAKOVIC, B., HABIBI, E., WANG, S. Y., ARTS, R. J. W., DAVAR, R., MEGCHELENBRINK, W., KIM, B., KUZNETSOVA, T., KOX, M., ZWAAG, J., MATARESE, F., VAN HEERINGEN, S. J., JANSSEN-MEGENS, E. M., SHARIFI, N., WANG, C., KERAMATI, F., SCHOONENBERG, V., FLICEK, P., CLARKE, L., PICKKERS, P., HEATH, S., GUT, I., NETEA, M. G., MARTENS, J. H. A., LOGIE, C. & STUNNENBERG, H. G. 2016. beta-Glucan Reverses the Epigenetic State of LPS-Induced Immunological Tolerance. *Cell,* 167**,** 1354–1368 e14.
72. Ogawa M, Yoshimori T, Suzuki T, Sagara H, Mizushima N, Sasakawa C (2005) Escape of intracellular Shigella from autophagy. Science 307:727–731
73. OHNO, H. (2016) Intestinal M cells. J Biochem 159:151–160
74. Olszak T, Neves JF, Dowds CM, Baker K, Glickman J, Davidson NO, Lin CS, Jobin C, Brand S, Sotlar K, Wada K, Katayama K, Nakajima A, Mizuguchi H, Kawasaki K, Nagata K, Muller W, Snapper SB, Schreiber S, Kaser A, Zeissig S, Blumberg RS (2014) Protective mucosal immunity mediated by epithelial CD1d and IL-10. Nature 509:497–502
75. Olszewski KL, Llinas M (2011) Central carbon metabolism of Plasmodium parasites. Mol Biochem Parasitol 175:95–103
76. Peterson LW, Artis D (2014) Intestinal epithelial cells: regulators of barrier function and immune homeostasis. Nat Rev Immunol 14:141–153
77. Petrova VN, Sawatsky B, Han AX, Laksono BM, Walz L, Parker E, Pieper K, Anderson CA, de Vries RD (2019) Incomplete genetic reconstitution of B cell pools contributes to prolonged immunosuppression after measles. Sci Immunol, 4
78. Pizarro-Cerda J, Cossart P (2004) Subversion of phosphoinositide metabolism by intracellular bacterial pathogens. Nat Cell Biol 6:1026–1033
79. Ratner JJ, Thomas VL, Sanford BA, Forland M (1981) Bacteria-specific antibody in the urine of patients with acute pyelonephritis and cystitis. J Infect Dis 143:404–412
80. REINER, N. E. (1987) Parasite accessory cell interactions in murine leishmaniasis. I. Evasion and stimulus-dependent suppression of the macrophage interleukin 1 response by Leishmania donovani. J Immunol 138:1919–1925
81. Reiner NE, Ng W, McMaster WR (1987) Parasite-accessory cell interactions in murine leishmaniasis. II. Leishmania donovani suppresses macrophage expression of class I and class II major histocompatibility complex gene products. J Immunol 138:1926–1932
82. Rice PJ, Adams EL, Ozment-Skelton T, Gonzalez AJ, Goldman MP, Lockhart BE, Barker LA, Breuel KF, Deponti WK, Kalbfleisch JH, Ensley HE, Brown GD, Gordon S, Williams DL (2005) Oral delivery and gastrointestinal absorption of soluble glucans stimulate increased resistance to infectious challenge. J Pharmacol Exp Ther 314:1079–1086
83. Rother M, Teixeira da Costa AR, Zietlow R, Meyer TF, Rudel T (2014) Modulation of Host Cell Metabolism by Chlamydia trachomatis. Microbiol Spectr, 7
84. Russell SL, Lamprecht DA, Mandizvo T, Jones TT, Naidoo V, Addicott KW, Moodley C, Ngcobo B, Crossman DK, Wells G, Steyn AJ (2019) Compromised Metabolic Reprogramming Is an Early Indicator of CD8(+) T Cell Dysfunction during Chronic Mycobacterium tuberculosis Infection. Cell Rep, 29 3564–3579 e5
85. Saeed S, Quintin J, Kerstens HH, Rao NA, Aghajanirefah A, Matarese F, Cheng SC, Ratter J, Berentsen K, van der Ent MA, Sharifi N, Janssen-Megens EM, ter Huurne M, Mandoli A, van Schaik T, Ng A, Burden F, Downes K, Frontini M, Kumar V, Giamarellos-Bourboulis EJ, Ouwehand WH, van der Meer JW, Joosten LA, Wijmenga C, Martens JH, Xavier RJ, Logie C, Netea MG, Stunnenberg HG (2014) Epigenetic programming of monocyte-to-macrophage differentiation and trained innate immunity. Science 345:1251086
86. Saluzzo S, Gorki AD, Rana BMJ, Martins R, Scanlon S, Starkl P, Lakovits K, Hladik A, Korosec A, Sharif O, Warszawska JM, Jolin H, Mesteri I, McKenzie ANJ, Knapp S (2017) First-Breath-Induced Type 2 Pathways Shape the Lung Immune Environment. Cell Rep 18:1893–1905

87. Sanchez EL, Lagunoff M (2015) Viral activation of cellular metabolism. Virology 479–480:609–618
88. Schiwon M, Weisheit C, Franken L, Gutweiler S, Dixit A, Meyer-Schwesinger C, Pohl JM, Maurice NJ, Thiebes S, Lorenz K, Quast T, Fuhrmann M, Baumgarten G, Lohse MJ, Opdenakker G, Bernhagen J, Bucala R, Panzer U, Kolanus W, Grone HJ, Garbi N, Kastenmuller W, Knolle PA, Kurts C, Engel DR (2014) Crosstalk between sentinel and helper macrophages permits neutrophil migration into infected uroepithelium. Cell 156:456–468
89. Shah-Simpson S, Lentini G, Dumoulin PC, Burleigh BA (2017) Modulation of host central carbon metabolism and in situ glucose uptake by intracellular Trypanosoma cruzi amastigotes. PLoS Pathog, 13 e1006747
90. Sherwood RK, Roy CR (2016) Autophagy Evasion and Endoplasmic Reticulum Subversion: The Yin and Yang of Legionella Intracellular Infection. Annu Rev Microbiol 70:413–433
91. Shi LZ, Wang R, Huang G, Vogel P, Neale G, Green DR, Chi H (2011) HIF1alpha-dependent glycolytic pathway orchestrates a metabolic checkpoint for the differentiation of TH17 and Treg cells. J Exp Med 208:1367–1376
92. Shih CJ, Wu YL, Chao PW, Kuo SC, Yang CY, Li SY, Ou SM, Chen YT (2015) Association between Use of Oral Anti-Diabetic Drugs and the Risk of Sepsis: A Nested Case-Control Study. Sci Rep 5:15260
93. Shin DM, Jeon BY, Lee HM, Jin HS, Yuk JM, Song CH, Lee SH, Lee ZW, Cho SN, Kim JM, Friedman RL (2020) Mycobacterium tuberculosis eis regulates autophagy, inflammation, and cell death through redox-dependent signaling. PLoS Pathog, 6 e1001230
94. Skokos D, Botros HG, Demeure C, Morin J, Peronet R, Birkenmeier G, Boudaly S, Mecheri S (2003) Mast cell-derived exosomes induce phenotypic and functional maturation of dendritic cells and elicit specific immune responses in vivo. J Immunol 170:3037–3045
95. Song J, Duncan MJ, Li G, Chan C, Grady R, Stapleton A, Abraham SN (2007) A novel TLR4-mediated signaling pathway leading to IL-6 responses in human bladder epithelial cells. PLoS Pathog, 3 e60
96. Soroosh P, Doherty TA, Duan W, Mehta AK, Choi H, Adams YF, Mikulski Z, Khorram N, Rosenthal P, Broide DH, Croft M (2013) Lung-resident tissue macrophages generate Foxp3+ regulatory T cells and promote airway tolerance. J Exp Med 210:775–788
97. Starr T, Child R, Wehrly TD, Hansen B, Hwang S, Lopez-Otin C, Virgin HW, Celli J (2012) Selective subversion of autophagy complexes facilitates completion of the Brucella intracellular cycle. Cell Host Microbe 11:33–45
98. Stephenne X, Foretz M, Taleux N, van der Zon GC, Sokal E, Hue L, Viollet B, Guigas B (2011) Metformin activates AMP-activated protein kinase in primary human hepatocytes by decreasing cellular energy status. Diabetologia 54:3101–3110
99. Svedberg FR, Brown SL, Krauss MZ, Campbell L, Sharpe C, Clausen M, Howell GJ, Clark H, Madsen J, Evans CM, Sutherland TE, Ivens AC, Thornton DJ, Grencis RK, Hussell T, Cunoosamy DM, Cook PC, Macdonald AS (2019) The lung environment controls alveolar macrophage metabolism and responsiveness in type 2 inflammation. Nat Immunol 20:571–580
100. Tannahill GM, Curtis AM, Adamik J, Palsson-Mcdermott EM, McGettrick AF, Goel G, Frezza C, Bernard NJ, Kelly B, Foley NH, Zheng L, Gardet A, Tong Z, Jany SS, Corr SC, Haneklaus M, Caffrey BE, Pierce K, Walmsley S, Beasley FC, Cummins E, Nizet V, Whyte M, Taylor CT, Lin H, Masters SL, Gottlieb E, Kelly VP, Clish C, Auron PE, Xavier RJ, O'Neill LA (2013) Succinate is an inflammatory signal that induces IL-1beta through HIF-1alpha. Nature 496:238–242
101. Thapa B, Lee K (2019) Metabolic influence on macrophage polarization and pathogenesis. BMB Rep 52:360–372
102. Traven A. Naderer T (2019) Central metabolic interactions of immune cells and microbes: prospects for defeating infections. EMBO Rep, 20, e47995.
103. Tsuji M, Komatsu N, Kawamoto S, Suzuki K, Kanagawa O, Honjo T, Hori S, Fagarasan S (2009) Preferential generation of follicular B helper T cells from Foxp3+ T cells in gut Peyer's patches. Science 323:1488–1492

104. Tucey TM, Verma J, Harrison PF, Snelgrove SL, Lo TL, Scherer AK, Barugahare AA, Powell DR, Wheeler RT, Hickey MJ, Beilharz TH (2018) Glucose Homeostasis Is Important for Immune Cell Viability during Candida Challenge and Host Survival of Systemic Fungal Infection. Cell Metab, 27, 988–1006 e7
105. Valle-Casuso JC, Angin M, Volant S, Passaes C, Monceaux V, Mikhailova A, Bourdic K, Avettand-Fenoel V, Boufassa F, Sitbon M, Lambotte O (2019) Cellular Metabolism Is a Major Determinant of HIV-1 Reservoir Seeding in CD4(+) T Cells and Offers an Opportunity to Tackle Infection. Cell Metab, 29, 611–626 e5
106. von Moltke J, Ji M, Liang HE, Locksley RM (2016) Tuft-cell-derived IL-25 regulates an intestinal ILC2-epithelial response circuit. Nature 529:221–225
107. Wang R, Dillon CP, Shi LZ, Milasta S, Carter R, Finkelstein D, McCormick LL, Fitzgerald P, Chi H, Munger J, Green DR (2011) The transcription factor Myc controls metabolic reprogramming upon T lymphocyte activation. Immunity 35:871–882
108. Wu S, Shen Y, Zhang S, Xiao Y, Shi S (2020) Salmonella Interacts With Autophagy to Offense or Defense. Front Microbiol 11:721
109. Wu XR, Kong XP, Pellicer A, Kreibich G, Sun TT (2009) Uroplakins in urothelial biology, function, and disease. Kidney Int 75:1153–1165
110. Yu X, Buttgereit A, Lelios I, Utz SG, Cansever D, Becher B, Greter M (2017) The Cytokine TGF-beta Promotes the Development and Homeostasis of Alveolar Macrophages. Immunity, 47, 903–912 e4
111. Zarrouk M, Finlay DK, Foretz M, Viollet B, Cantrell DA (2014) Adenosine-mono-phosphate-activated protein kinase-independent effects of metformin in T cells. PLoS One, 9, e106710
112. Zhang X, Zhao S, Sun L, Li W, Wei Q, Ashman RB, Hu Y (2017) Different virulence of candida albicans is attributed to the ability of escape from neutrophil extracellular traps by secretion of DNase. Am J Transl Res 9:50–62
113. Zhou G, Myers R, Li Y, Chen Y, Shen X, Fenyk-Melody J, Wu M, Ventre J, Doebber T, Fujii N, Musi N, Hirshman MF, Goodyear LJ, Moller DE (2001) Role of AMP-activated protein kinase in mechanism of metformin action. J Clin Invest 108:1167–1174
114. Zhou L, Lopes JE, Chong MM, Ivanov II, Min R, Victora GD, Shen Y, Du J, Rubtsov YP, Rudensky AY, Ziegler SF (2008) TGF-beta-induced Foxp3 inhibits T(H)17 cell differentiation by antagonizing RORgammat function. Nature 453:236–240

Metabolic Reprogramming and Cancer

Ana Paula Lepique, Enrique Boccardo, and Flávia Sardela de Miranda

1 A Brief Historical Perspective

Cancer cells display several metabolic alterations frequently described as "Warburg effect". In 1953, the German scientist, Otto Warburg, published a paper entitled "On the origin of cancer cells" [1]. In this paper, he described observations that led him to the theory that tumor cells developed as a consequence of two key events. First, an injury to cell respiration, which could be caused by different factors such as hypoxia or drugs that inhibited or uncoupled the oxidative phosphorylation, which ultimately damaged the mitochondria and therefore energy production through the electron transport chain. Secondly, the cells, through a proliferation dependent selection process, increased fermentation, which led to the transformed phenotype [1]. This idea started with the observation that tumor cells could proliferate in hypoxic environments and maintained the same metabolic profile even if cultured in normoxia. The idea that metabolic changes caused the transformed cell phenotype has been under discussion for a long time. Studies from some research groups have produced data that support the existence of a causal relationship between mutations in homoplasmy in mitochondrial genes and cell transformation. However, this is not the accepted paradigm for the majority of cancers. Although, there is evidence that for a few types of cancer loss of function of proteins related to oxidative phosphorylation has a causal relationship with carcinogenesis. This issue will be addressed in detail later in this chapter [2, 3]. There are several evidences showing that mutations in proto-oncogenes and tumor suppressor genes modulate cell metabolism and do not necessarily involve genetic changes in genes related to the oxidative phosphorylation. For instance, Ras, Akt and Myc activity may promote, through various mechanisms, glycolysis while inhibiting oxidative phosphorylation. Moreover, loss of p53 function, which is a common event in tumors, can also lead to an increase in

A. P. Lepique (✉) · E. Boccardo · F. S. de Miranda
Instituto de Ciências Biomédicas, Universidade de São Paulo, Sao Paulo, Brazil
e-mail: alepique@icb.usp.br

N. O. S. Camara et al. (eds.), *Essential Aspects of Immunometabolism in Health and Disease*, https://doi.org/10.1007/978-3-030-86684-6_9

glycolysis. Another important factor that has to be taken into account is the role of the tumor microenvironment on cells behavior and metabolism. The tumor microenvironment is complex and is composed by a range of different niches according to pH, oxygen and nutrient availability, nature of infiltrating cells and other factors. Therefore, within the tumor microenvironment metabolic demands vary and may modulate the tumor cell metabolism. We will explore each of these factors through this chapter and finish by exploring the effects tumor cells exert over leukocytes and immune responses.

2 Cancer Cells

Cancer is a disease characterized by abnormal cell proliferation and the ability of tumor cells to invade other tissues in the body. Carcinogenic mechanisms can be direct or indirect. Genetic and epigenetic alterations that lead to proto-oncogene gain of function or tumor suppressor genes inactivation can directly initiate a carcinogenic process. Chronic inflammation causing repeated cycles of tissue injury and repair, oxidative stress, cytokine and growth factor secretion, can in time, also initiate a carcinogenic process. The carcinogenic process involves the accumulation of various genetic and epigenetic alterations. The time frame during which this occurs is usually long and the cells involved are submitted to selective pressure at all times. Among the main forces opposing tumor onset are the selective pressure from the immune system, natural competition for nutrients between actively growing cells, and intrinsic mechanisms that should lead to cell death upon cellular injury to warrant tissue homeostasis.

Cancers are composed by a heterogenous cell population containing tumor and stromal cells. Within the tumor cell population itself, there are subpopulations that exhibit different genetic profiles. This can be translated in different phenotypes characterized by diverse proliferative capacity, variable self-renewal potential, mobility and invasion capacity. Besides, there are many other cellular elements present in solid tumors. Endothelial cells, fibroblasts, leukocytes with anti-tumor phenotype as cytotoxic cells, leukocytes with pro-tumoral phenotype as alternative macrophages and regulatory T cells are also present in most tumors. Although not transformed, these cells play an essential role in tumor progression and fate [1].

2.1 Oncogenes and Tumor Suppressor Genes

Oncogenes of cellular origin are derived from activating mutations, gene amplification, translocation or other alterations that confer gain of function to normal cellular proto-oncogenes. Oncogenes drive cell cycle progression in an exaggerated manner, causing excessive proliferation which eventually favors cell transformation. Oncoproteins, coded by oncogenes, can be growth factors, growth factor receptors acting

via tyrosine kinase activity, cytoplasmic and membrane associated tyrosine kinases, serine/threonine protein kinases, GTP-binding proteins, transcription factors, or any protein with functions related to promote cellular growth, proliferation or inhibition of other proteins that negatively control the cell cycle. For example, some types of lymphoma can be triggered by the translocation that joins the immunoglobulin promoter to the c-myc coding region. This alteration leads to high constitutive c-Myc expression in the cells, which can drive transformation. The genetic alterations that cause a proto-oncogene change to oncogene are dominant (the alteration in one allele is enough to cause phenotypic changes in the cells). Oncogenes can also have viral origin. Several viruses can drive human cell transformation, through the expression of *bona fide* viral oncogenes in the host cell. This is the case of E6 and E7 oncoproteins from high-risk Human Papillomavirus. These viral factors promote, among other functions, the degradation of p53 and Retinoblastoma, pRb, tumor suppressor proteins, which are fundamental, respectively, for the maintenance of cellular homeostasis.

Tumor suppressor genes code the tumor suppressor proteins, which can control the cell cycle and even trigger apoptosis in cells with genomic alterations or unscheduled cell cycle progression due to deregulated checkpoints (see below). Usually, the expression of both alleles of tumor suppressor genes needs to be altered, by mutation or other mechanism, for the phenotype to be expressed. Examples of tumor suppressor genes are the retinoblastoma protein, p53 and BRCA1 (BReast CAncer 1) [4].

Besides the genetic and epigenetic alterations mentioned before, miRNAs are also important regulators of gene expression, through translation repression. Experimental evidence of decades of research have shown that miRNAs are directly involved in several types of human cancer, displaying roles as oncogenes or tumor suppressors. Overexpression of some miRNAs can promote cancer development by inhibiting translation of tumor suppressor genes, genes involved in cellular differentiation and apoptosis triggering. Similarly, under expressed miRNAs may promote increased expression of genes that promote cell proliferation, glycolytic metabolism and inflammation. Examples of such miRNAs are miRNA 17–92 that downregulates PTEN and pRB, while miRNA-155 upregulates c-Myc [5].

2.2 Cell Cycle Control

Most cells in an adult organism are quiescent and perform their functions in a non-proliferative state. Exceptions to this rule are cells from tissues that must be replenished as bone marrow, skin, mucosa and other populations with self-renewal capacity. The quiescent cells are in what is called G0 phase of the cell cycle, which means they are not proliferating, and are simply maintaining their homeostasis and executing their function in the tissue. Upon mitogenic stimulation, however, cells pass through the cell cycle to generate two daughter cells. The cell cycle is divided in 4 phases: G1, S, G2 and mitosis. The 3 first phases of the cell cycle, which correspond to the

interphase, prepare the cells for the division that happens during mitosis. During the G1 phase, or gap 1, cells accumulate mass, synthetizing organelles, biomolecules and preparing for DNA synthesis. DNA replication happens during the S phase. During the second gap phase, G2, cells gain more mass, more organelles are synthetized and organized in the intracellular space while the cell prepares for mitosis. G2 is followed by the mitotic phase, which leads to the generation of two daughter cells (Fig. 1).

The biochemical signals that trigger cell cycle usually activate cell membrane tyrosine kinase receptors. Mitogenic stimuli reorganize the cellular transcription program through signaling pathways such as Ras/Raf/MAPK, PI3K/Akt, NFkB, etc. The first genes to be expressed are known as immediate early genes (transcription independent of protein translation). Some of the products of the immediate early genes are transcription factors that will control the second gene expression wave. Among these genes, c-myc plays a central role in cell growth. Cells need to grow, gain mass and replicate organelles before they are committed to the duplication of the genome, in order to generate daughter cells. Another important pathway that promotes cellular growth is the PI3K/Akt/mTOR pathway (Phosphatidyl inositol 3 kinase/Akt/mammalian Target of Rapamycin), which leads to cellular proliferation and protein synthesis, among other effects. The cellular growth during the G1 phase

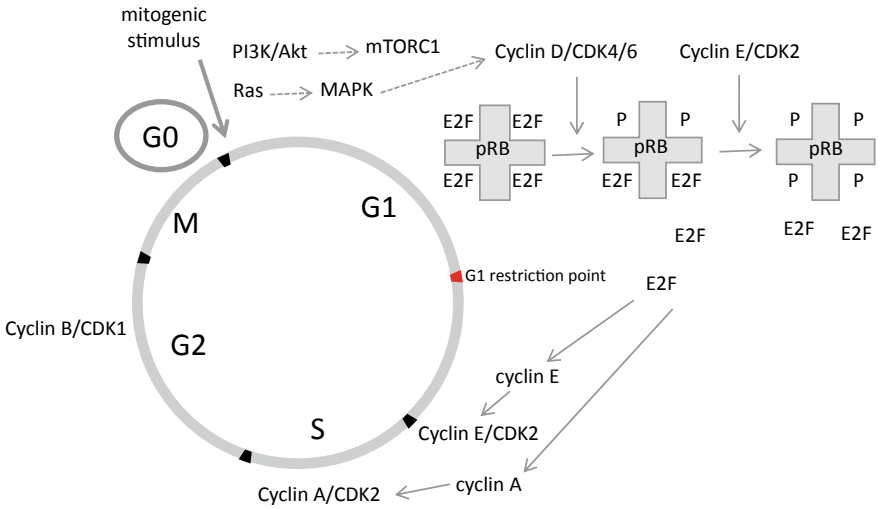


Fig. 1 Cell cycle phases and molecular steps controlling G1 progression. Upon mitogenic stimulation, through receptors that lead to activation of signaling pathways as Ras/MAPK and PI3K/Akt/mTOR, gene expression will eventually include cyclin D as a target. Cyclin D dimerizes with CDK4 or 6. This complex phosphorylates pRB, releasing E2F that mediates the expression of cyclin E and cyclin A. The proteins translated from these genes dimerize with CDK2. Several genes related to DNA synthesis will then be expressed and cells can continue the cell cycle. The restriction point, indicated in red, marks the G1 checkpoint where cells must have nutrients and mass to be able to commit to DNA synthesis

of the cell cycle is checked at the restriction point near the transition between G1 and S phases. This is an essential cell cycle checkpoint that is mainly regulated by the antiproliferative activity of the hypophosphorylated form of the tumor suppressor pRB. Molecularly, gene expression triggered by mitogenic factors induces the expression of Cyclin D (CycD), the regulatory partner of CDK4 (Cyclin Dependent Kinase 4). Once formed, the CycD/CDK4 complex phosphorylates pRb. This releases the transcription factors E2F that, in turn, stimulates the expression of several genes related to DNA synthesis including Cyclin E and Cyclin A, that will mediate the next cell cycle phases. Cyclin E will form a complex with CDK2 further phosphorylating pRb and pushing the cell cycle through the G1/S transition, while CycA/CDK2 complex will drive the S phase (Fig. 1). The G1 checkpoint, therefore, guarantees that daughter cells will be viable regarding function. As mentioned before, cancer is characterized by continuous unchecked cellular proliferation. It is common to observe that cancer cells display shorter G1 phase than normal cells. Nevertheless, they are still able to accumulate enough mass to overcome the restriction point in G1. Tumor cells are able to gain mass even with faster proliferation rates than other cells due to metabolic changes that were first described by Otto Warburg. Tumor cells behave as cells in hypoxia, using glycolysis for energy production, even when growing at normal oxygen levels. The use of glycolytic metabolism allows cells to change pathways to anabolism and macromolecules synthesis. Tumor cells, however, do not rely only on glycolysis, as will be shown later in this chapter; they can use different sources or nutrients and use more than one metabolic pathway to maintain energy levels and anabolic activity for proliferation [6].

2.3 Cancer Cell Metabolism

As briefly mentioned before, tumor cells display altered metabolism compared to normal cells. In normal cells, in the presence of oxygen, each glucose molecule processed through the glycolytic pathway generates 2 pyruvate molecules that are oxidized to 2 acetyl-Coenzyme A molecules to integrate the tricarboxylic acid cycle (TCA) to produce reduced co-factors NADH (reduced nicotinamide adenine dinucleotide) and FADH2 (dihydroflavine-adenine dinucleotide). These co-factors donate their high-energy electrons to specialized protein complexes present in the inner mitochondria membrane. These protein complexes function as proton pumps and their activity generates a proton gradient between the inner and outer mitochondria membranes. This pathway is known as the oxidative phosphorylation pathway, and its final step involves the transport of a proton through the inner membrane back to the interior of the mitochondria by the ATPse pump. This process releases enough potential energy to allow for an inorganic phosphate to bind to ADP, generating ATP and CO_2, at the expense of O_2 and H_2O. Using this metabolic pathway, a cell can produce 38 mol of ATP per mol of glucose. Cells also recycle oxidized co-factors to be reduced in the TCA cycle, also in a mechanism indirectly dependent on molecular oxygen.

The Warburg effect postulates that tumor cells preferentially use the glycolytic pathway, even in normoxia (Fig. 2). Today, we understand that the cancer cell metabolism is plastic and adaptable to variations in the microenvironment conditions. Cancer cells can use different substrates as sources of energy and building blocks for macromolecules including glucose, glutamine, fatty acids and even lactate, as will be explored later in this chapter.

It has been shown that oncogenes are able to drive aerobic glycolysis, where tumor cells consume 1 mol of glucose to generate 4 mol ATP through the glycolytic pathway producing lactate, without production of pyruvate to nourish the TCA. Therefore, while normal cells preferentially use glycolysis, the tricarboxylic acid cycle and oxidative phosphorylation for ATP synthesis, tumor cells preferentially use the glycolic pathway pyruvate and lactate as an end product (Fig. 2). The net

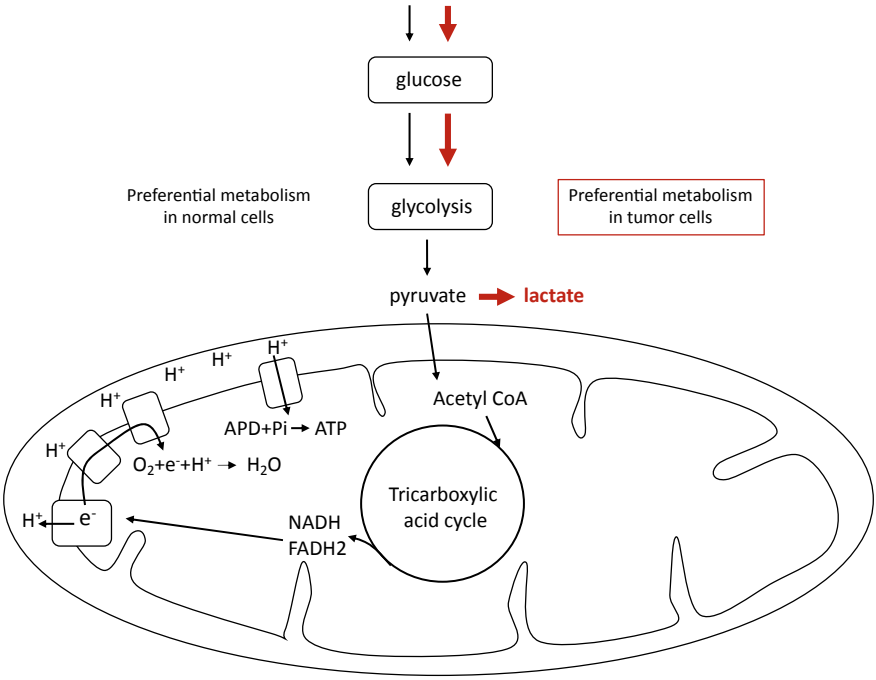


Fig. 2 Normal and cancer cells exploit different pathways for ATP synthesis. The scheme shows a comparative description of the metabolic pathways used by normal (black) and tumor cells (red) for ATP synthesis. Normal cells use the pyruvate molecules to produce Acetyl CoA, which is completely oxidized by the tricarboxylic acid (TCA) cycle and oxidative phosphorylation. NADH and FADH2, reduced in the TCA cycle, are donors of electrons and protons to the complexes present in the mitochondria inner membrane. These complexes pump protons, forming a proton gradient between the two mitochondrial membranes, and transport electrons from complex I to III to IV, where the electrons are combined with oxygen generating H_2O. The protons are pumped back into the mitochondria through complex V, a process that releases enough energy to allow for the binding of an inorganic phosphate to ADP to generate ATP. Cancer cells increase the conversion of pyruvate molecules to lactate, which is exported to the extracellular compartment

ATP production from the last pathway is just a fraction of the amount produced by the former. To compensate for this deficiency, tumor cells display high glucose uptake, mediated by the glucose transporters GLUT. The increased uptake of glucose by tumor cells has been used as a marker for detection of very small tumor masses in the body, through positron emission tomography (PET) after injection of 2-(^{18}F)-fluoro-2-deoxy-D-glucose (FDG) in cancer patients [7].

The increase in glucose uptake is paralleled by higher glycolytic pathway activity due to the upregulated expression of key enzymes in cancer cells. For instance, hexokinase (HK) is usually upregulated in cancer. This enzyme catalyzes the first irreversible step of glycolysis, the phosphorylation of glucose, a necessary step for glycolysis. There are four isoforms of HK, and HK2 is the main enzyme involved in cancer cell metabolism. It is expressed in the mitochondrial membrane, associated to the pore-like outer mitochondrial membrane protein voltage-dependent anion channel (VDAC), the inner mitochondrial membrane protein ATP synthase (ATP is another substrate to HK) and the adenine nucleotide translocator that transports ATP to the VDAC/HK-II complex. The association with this complex warrants HK2 access to ATP to allow efficient phosphorylation of glucose. There is data showing HK-II gene amplification in cancer cells. Moreover, there are several regulatory elements in the HK-II promoter responsive to HIF-1, Ras/PKC, p53 and other signaling pathways [7].

The fact that tumor cells are selected to use a less efficient metabolic pathway to produce energy may seem counterintuitive. However, this strategy confers some advantages. For instance, cancer cells can survive in low oxygen tension, which is a common condition in several tumor areas. Besides, the products of glycolysis can suppress immune responses and they also can facilitate tumor cell invasion, as will be addressed later in this chapter. The extracellular acidic pH, which results from the secretion of lactate, and lactate itself stimulate angiogenesis, while stromal cells uptake lactate, through monocarboxylate transporters, and can generate pyruvate that is used as carbon source in oxidative phosphorylation. This results in a mixed metabolic profile between tumor and stromal cells which allows cell survival and proliferation. The shift in the use of these metabolic pathways allows the synthesis of macromolecules necessary for cell growth and proliferation. Finally, but also very important, the metabolic shift can result in production of NADPH to equilibrate the redox status in tumor cells.

A metabolic pathway closely connected to glycolysis is the pentose phosphate pathway (PPP). This pathway is divided in oxidative and non-oxidative phases. In the oxidative phase, 5 mol of glucose-6P are converted in 6 mol ribulose-5P. Ribulose-5P is converted to ribose-5P, which through a series of reactions generates fructose-6P and glyceraldehyde-3P, which are intermediates of the glycolytic pathway. Depending on the metabolic demand, the PPP pathway can be directed toward production of NADPH and ribose-5P, or can favor glycolysis and pyruvate generation. In tumor cells, the pentose phosphate pathway is important for various reasons. First, it generates NADPH and ribose-5P, which are precursors for fatty acids and nucleotide synthesis, respectively. Second, as previously mentioned it can

generate glycolysis intermediates. Third, NADPH generated in this pathway is important for oxidative stress protection, which is important in all cells, but particularly in tumor cells. Therefore, in tumor cells, part of the glucose is directed to the PPP as means of production of macromolecules critical for cell growth and DNA synthesis, as well as for protection against oxidative stress (Fig. 3) [8, 9].

Besides those involved in the pathways described above, other glycolytic intermediates are also used by tumor cells for synthesis of macromolecules. Important representative examples are dihydroxyacetone that is involved in triacyl glyceride and phospholipid synthesis and pyruvate that is used as a precursor for alanine and malate synthesis. These processes are favored by the expression of the embryonic

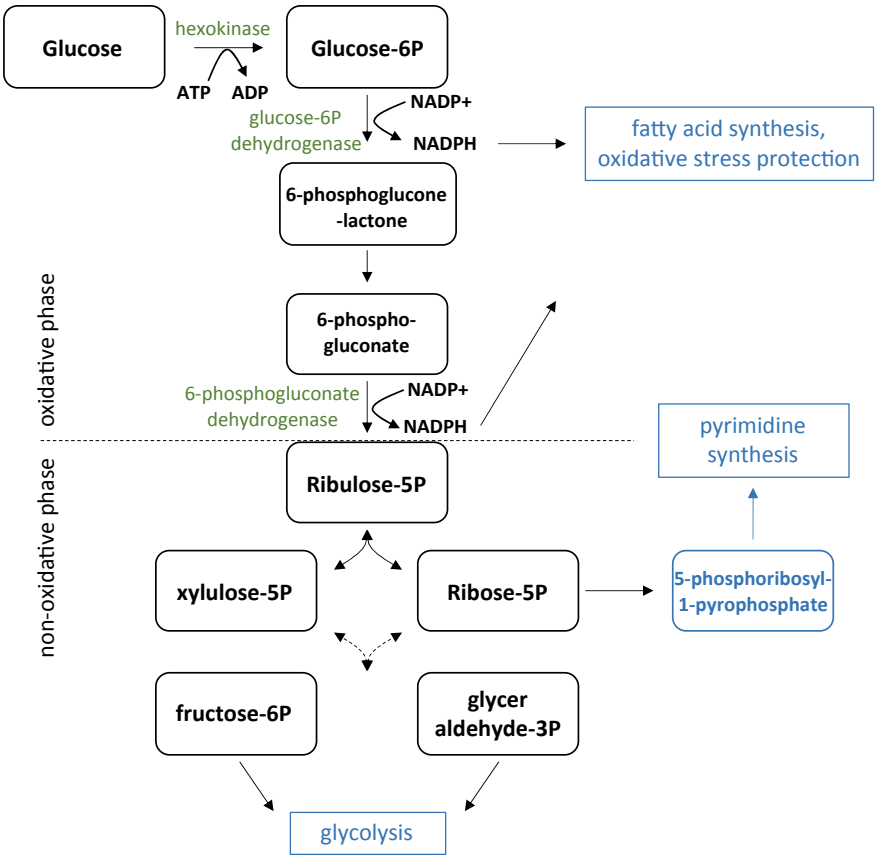


Fig. 3 Schematic representation of the pentose phosphate pathway (PPP). This metabolic pathway uses glucose to generate NADPH and pyrimidine precursors, necessary for cell growth and DNA synthesis. Besides, NADPH is important for protection against oxidative stress. Oxidative and non-oxidative phases of the pathway are represented, as well as enzymes (in green) that regulate irreversible reactions. Biological processes downstream to PPP are represented in blue

isoform of pyruvate kinase, PKM2, in tumor cells. This isoform exhibits low enzymatic activity leading to the accumulation of glycolytic intermediates and increase the concentration of substrates for anabolic reactions. This includes phospholipid, serine and nucleotide synthesis and lactate reduction (Fig. 4). Actually, PKM2 also increases the PPP activity through the accumulation of glycolysis intermediates. PKM2 activity seems to increase due to post-translational mechanisms, as tetramerization and phosphorylation, rather than increase in expression [8]. The important role of PKM2 in cancer is highlighted by the fact that this enzyme has several non-metabolic functions, most of them associated with the regulation of gene expression [4].

The TCA cycle is also altered in tumor cells. In normal cells, Acetyl-CoA is a carbon source leading to the synthesis of the various intermediates of the cycle and to the reduction of NADH and FADH2. In turn, these co-factors donate electrons and

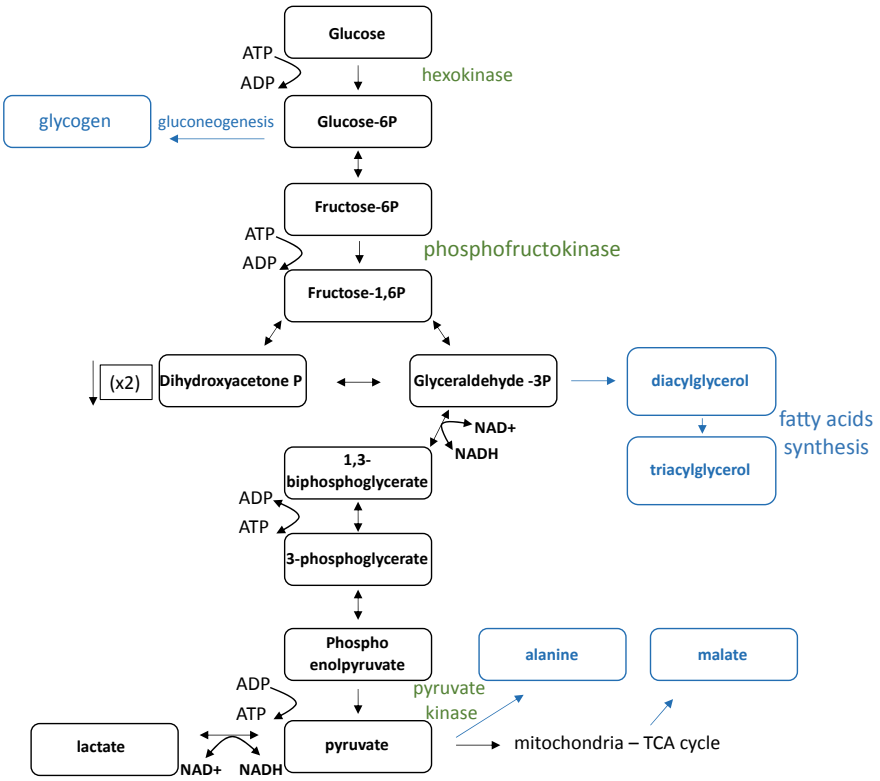


Fig. 4 Schematic view of the glycolysis pathway (black) and anabolic reactions that use glycolytic intermediates as substrates (blue). TCA cycle corresponds to the tricarboxylic acid cycle. Enzymes depicted in green catalyze regulatory reactions in this pathway. In blue are downstream processes associated with the indicated metabolites

protons to the oxidative phosphorylation pathway. However, in tumor cells, Acetyl-CoA is not properly incorporated into the TCA. Its excess is transported to the cytoplasm, where it is used as substrate for fatty acids, cholesterol and isoprenoids synthesis. Although many tumor cells preferentially use the glycolytic pathway, data in the literature shows that many tumors can use both aerobic glycolysis and oxidative phosphorylation. Indeed, most tumor cells have functional oxidative phosphorylation pathway. Interestingly, when tumor cells use the TCA, the pathway has mostly normal function, but also has a role in amino acids and lipid synthesis. Cells in which these pathways operate simultaneously, can use glutamine as well as Acetyl CoA from the fatty acids β-oxidation as substrates of the TCA cycle (Fig. 5).

There is a general idea that in many tumors the tricarboxylic acid (TCA) cycle is truncated. However, available evidence supports that the TCA cycle is actually aberrant. In fact, several TCA reactions do take place in cancer cells depending on the substrates and mechanisms of uptake that are available. In this way, metabolic pathways can work together to generate both the energy and macromolecules necessary for sustained cell proliferation and to control redox conditions. The TCA is a central hub in normal cell metabolism, taking place in the mitochondrial matrix, and feeding reduced co-factors for the oxidative phosphorylation pathway, as well as metabolic intermediates to several processes in the cells. In cancer cells, glucose is shunt from the TCA cycle. However, in these conditions glutamine and fatty acids can be used as sources for the TCA (Fig. 5). Actually, recent data has indicated that tumor cells can be addicted to glutamine, relying heavily in the TCA cycle [11]. These cells can also use fatty acids to feed intermediates to this cycle, while still using aerobic glycolysis and generating lactate. Glutamine is the most abundant amino acid in the human body. It plays a role in transporting nitrogen to cells and organelles. Glutaminolysis is the process by which enzymes catalyze the conversion of glutamine to glutamate and then α-ketoglutarate (α-KG), which in turn is incorporated in the TCA. Therefore, glutamine is used not only as an energy source, through the TCA cycle and oxidative phosphorylation, but also for as a source of carbon backbone for the synthesis of several macromolecules, including non-essential amino acids, purines and pyrimidines. The importance of glutamine in cancer cells biology is highlighted by the fact that glutamine transporters and enzymes that catalyze glutaminolysis are up regulated in cancer cells, in a c-Myc dependent manner [10]. Interestingly, some tumor cells lines, for instance HeLa, which uses both glycolysis and oxidative phosphorylation, grow better in high glutamine concentration [11].

Another pathway reprogrammed in cancer cells and that contributes to the TCA cycle is fatty acid oxidation or β-oxidation. Fatty acids are the building blocks of triglycerides, phospholipids and sphingolipids. These lipids are important components of cellular and organelles membranes and, therefore, essential for proliferating cells. Besides, these molecules perform important functions acting as second messengers and energy storage. Tumor cells may synthetize fatty acids de novo from glucose, acetate and amino acids, as glutamine. However, cells can also uptake exogenous fatty acids and store them in lipid droplets. Upon need, these lipids can be used, either as sources for synthesis or energy, through the β-oxidation pathway, in an oxygen

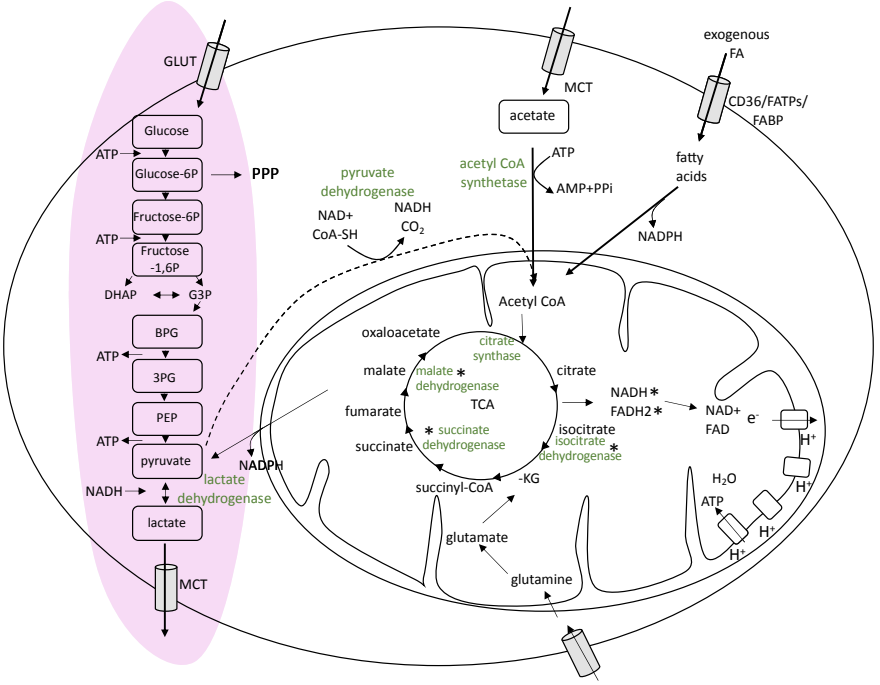


Fig. 5 Multiple metabolic pathways operate simultaneously to produce energy and macromolecules to support tumor cells growth. While tumor cells increase their glucose uptake (which is metabolized through aerobic glycolysis—pink), through upregulation and cell membrane localization of GLUT (glucose transporters), they can also uptake glutamine through one of the SCL transporters (soluble carrier family), exogenous fatty acids (FA) and acetate. While acetate can be directly converted in Acetyl CoA, the β-oxidation of fatty acids generates several Acetyl CoA molecules that can be promptly incorporated in the TCA. Glutamine is converted to glutamate, which is converted in α-ketoglutarate (α-KG), which then enters the TCA cycle to generate macromolecules and energy, through a process called glutaminolysis. Malate from the TCA can also be exported to the cytosol and converted to pyruvate through a reaction catalyzed by the NADP-dependent form of malic enzyme, also leading to NADP reduction, which is used for anabolism of macromolecules. Glutamine also can contribute with one carbon for de novo fatty acids synthesis. And finally, NADH and FADH2 (steps that reduce these cofactors are indicated by *) generated in the TCA cycle donate electrons for the oxidative phosphorylation pathway (yellow) to generate ATP. Together glutaminolysis (blue), glycolysis (pink) and β-oxidation can contribute to both energy generation and anabolism for cell growth and proliferation. Enzymes catalyzing the main reactions in glycolysis and TCA are displayed in green. 3PG—3-phosphoglycerate; BPG—biphosphoglycerate; DHAP—dihydroxyacetone; G3P—glyceraldehyde-3-phosphate; MCT—monocarboxylate transporter; FATP—fatty acid transport proteins; FABP—fatty acid binding protein; PEP -phosphoenol pyruvate

dependent process. Different transporters can uptake fatty acids from the extracellular environment. As such, it is not surprising that the expression of some of them is upregulated in cancer cells. In fact, there is indication that high expression levels of CD36, one of the main proteins involved in fatty uptake in many cell types is a poor prognostic for different types of cancer [12].

β-Oxidation is also a source of acetyl CoA. This molecule enters the TCA cycle to generate reduced NADH and FADH2, which donate electrons to the oxidative phosphorylation pathway to generate ATP through oxidative phosphorylation. This is especially important in conditions of metabolic stress common in tumor microenvironment. Moreover, the β-oxidation pathway also generates NADPH, which is essential for synthesis of biomolecules and important for protection against oxidative stress. Interestingly, obesity is a risk factor for cancer. This is particularly true for renal, gastric, breast and colon carcinomas, which are tumors that grow in adipose tissue rich environments. Macrophages present in adipose tissue from obese patients display a pro-inflammatory phenotype, secreting of IL-6, IL-8, TNFα and VEGF, which can contribute with tumor progression. This in addition to the ready source of fatty acids presented by the adipocytes indicates how obesity can be a risk factor for cancer. In a hypoxic environment, there is little generation of Acetyl CoA from glucose. Under these circumstances, an exogenous source of fatty acids is important to sustain tumor cells growth. However, it is important to note that activated oncogenes can accelerate fatty acid uptake even in cells growing in normal oxygen concentrations.

Tumor cells also synthetize fatty acids, as well as phospholipids, and other important lipids for membrane synthesis and signaling. Data in the literature shows that oncogenes' activity induce the expression of enzymes involved in lipid biosynthesis. However, the patterns are heterogeneous and variable depending on the tumor type. The important point is that, depending on nutrients and oxygen availability and biochemical signaling by oncogenes, tumor cells can synthetize lipids, uptake them from exogenous sources, store and use these molecules to drive cell growth [13, 14].

Amino acids metabolism is also altered in cancer cells. One of the main alterations is in serine synthesis. This amino acid plays a key role in the synthesis of several macromolecules. For instance, serine is a precursor for the synthesis of glycine and cysteine. Glycine is a precursor for porphyrins and is incorporated into purine nucleotide bases. Besides, glycine is used in glutathione (GSH) synthesis, which is important for protection against oxidative stress, and participates in folate metabolism. Conversion of serine to glycine donates one carbon unit to tetrahydrofolate, which is then used in thymidine synthesis. The enzyme that catalyzes this reaction is serine hydroxylmethyltransferase (SHMT), which is a transcriptional target of the proto-oncogene c-Myc. The conversion of serine to glycine is coupled to the folate and methionine cycles, which in turn generate reactive intermediates that provides methyl-groups for the methylation of histones or DNA, and precursors for glutathione and nucleotides biosynthesis. Finally, serine also plays a key role in the synthesis of sphingosine and phospholipids. The main pathways involved in serine metabolism are depicted in Fig. 6.

Cells can obtain serine either by uptake from the extracellular environment or synthetize it from glucose. The last strategy seems to be important for cancer cells. Serine is synthetized by cells from 3-phosphoglycerate, a glycolysis intermediate, in a pathway called serine synthesis pathway, SSP (Fig. 6). The first reaction in this pathway is catalyzed by phosphoglycerate dehydrogenase (PHGDH). The gene that codes this enzyme is located in chromosome 1p12, within a region amplified in a

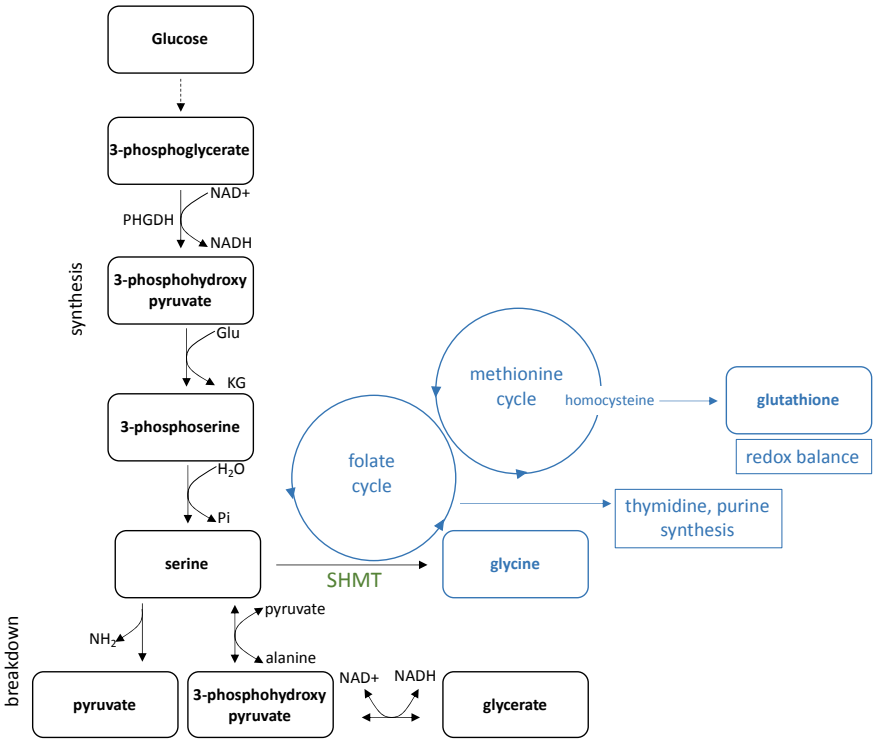


Fig. 6 Serine synthesis pathway and breakdown and link to folate and methionine cycles and consequences of activation. PHGDH—phosphoglycerate dehydrogenase; Glu—glutamate; α-KG—alpha ketoglutarate; SHMT—serine hydroxylmethyltransferase

percentage of human melanomas and breast cancers. PHGDH is also upregulated by transcription factors as c-Myc and ATF4. ATF4 activation of PHGDH is a downstream effect of NFR2 antioxidant transcription factor and also of mTORC1 complex [15]. This indicates that activation of serine synthesis pathway is important for protection against oxidative stress and amino acid deficiency. In some types of cancer, high expression of PHGDH is associated with higher proliferation or higher tumor grade (triple negative breast cancer and gliomas, respectively) [16].

Cancer cells can use the oxidative phosphorylation in different levels. While some cancer cells rely mainly on glycolysis for ATP production, there are reports of cancer cells that can use both glycolysis and oxidative phosphorylation and cells that even increase the function of the last. Regarding the cells that actually rely also or mainly in oxidative phosphorylation for energy, there is a hypothesis that proposes that at a first moment, oncogenes or other alterations inhibit oxidative phosphorylation, and in a second wave, there would be recovery of oxidative phosphorylation by glutamine uptake and glutaminolysis. In this scenario, tumor cells would use both glucose and glutamine, which actually happens in a number of cancer types. Eventually, glutamine would become the main nutrient source for both energy and anabolism. In this model,

the Warburg effect would represent just a phase during cancer progression. Another hypothesis that has gained space in the literature is the symbiotic relationship between tumor cells, which will be better discussed later. Briefly, in this scenario, tumor cells from hypoxic regions strongly relies on glucose and produces lactate, which is then used by the oxygenated tumor cells as a carbon source for the oxidative phosphorylation, increasing the availability of glucose for the hypoxic cells. This model also leaves room to understand how cancer cells can adapt to different environments and nutrient availability [17]. On the other hand, the mitochondrial genome encodes 13 polypeptides that take part in oxidative phosphorylation. The mitochondrial DNA is susceptible to mutations caused by the production of reactive oxygen species, which are a necessary by product of metabolism itself. Mutations in mitochondrial DNA, both in coding and regulatory regions, have been associated with several types of cancer as thyroid, colon, ovary and others. Therefore, mutations with loss of function of proteins important of oxidative phosphorylation may play a part in metabolic reprograming in cancer [2].

3 Microenvironment and Signaling Pathways Regulating Cancer Cell Metabolism

All of the metabolic pathways described above are controlled by biochemical signals triggered by oncoproteins activity, lack of function of tumor suppressor proteins, and microenvironment conditions as hypoxia and nutrient availability. This section will summarize the main signaling pathways controlling tumor cell metabolism.

The tumor microenvironment is heterogeneous in composition. First, the whole tumor structure is composed by different tumor cells clones. Second, other cell types, including several types of inflammatory cells, infiltrate the tumor parenchyma. Third, different biochemical and physical conditions can be readily detected in different regions of the tumor mass. Tumor blood vessels are disorganized and “leaky”, therefore inefficient to transport nutrients and oxygen to tumor cells, especially to those located in the most internal parts of the tumor. Indeed, most solid tumors exhibit hypoxic areas, while pH gradients, that vary according to tumor cell metabolism, can be observed. These alterations in the microenvironment are the result of intrinsic characteristics of the tumor cells themselves. Oncoproteins drive a series of changes in cells homeostasis, contributing to metabolic reprograming. The same can be said for alterations in tumor suppressor proteins activity or lack of it, as is the case of TP53.

3.1 HIF-1

One of the main regulators of cellular metabolism is the Hypoxia Induced Factor 1. This is a transcription factor, composed of two subunits, alpha and beta (Aryl hydrocarbon receptor nuclear translocator—Arnt). HIF1α is the regulatory subunit, and its activity is controlled mainly at the post-translational level. Proline residues in HIF1α are hydroxylated by prolyl-hydroxylases, PHDs, and target this factor for proteasomal degradation by the E3 subunit of the 26S proteasome complex containing the von Hippel-Lindau (pVHL) tumor suppressor protein. The HIF-1 transactivating activity can also be inhibited by FIH-1 (Factor Inhibiting HIF-1). This protein suppresses HIF-1 interaction with the histone acetyltransferase p300/CREB binding protein (CBP). As oxygen concentration drops in hypoxic conditions, both PHDs and FIH-1 are inhibited, leading to HIF-1 stabilization and activation. HIF-1 binds to the hypoxia-response elements, present at enhancers and promoter regions of a series of hypoxia responsive genes. HIF-1 can also be activated by cellular factors, or non-canonical mechanisms. Aberrant activation of the PI3K (phosphatidylinositol-3 kinase)/Akt pathway can induce HIF-1α transcription. Moreover, deficiency of the pVHL protein decreases ubiquitination of HIF-1α promoting its stabilization and increased activity. The same phenomenon is observed in the case of deficiency of other proteins involved in proteasome activity. The activity of both PHD and FIH-1 require α-ketoglutarate (α-KG) as well as oxygen. Therefore, alterations in the TCA cycle, due to mutations in succinate dehydrogenase or fumarate hydratase, leading to decrease α-KG concentration, can also lead to stabilization of HIF-1α. These non-canonical activation mechanisms can take place even in normoxic conditions.

Glucose transporter 1 (GLUT-1) is an important HIF-1 target since it allows tumor cells to increase glucose uptake. HIF-1 also increases the expression of lactate dehydrogenase, which catalyzes conversion of pyruvate to lactate, which characterizes aerobic glycolysis in normoxic regions and has an important effect on pH. Monocarboxylate transporter 4 (MCT4) is also a transcriptional target of HIF-1. This is one of the proteins responsible for the efflux of lactate to the extracellular environment, a process accompanied by the transport of a proton causing a decrease in the extracellular pH. At the same time, HIF-1 suppresses mitochondrial function. As mentioned before, pyruvate is converted to Acetyl CoA in a reaction catalyzed by pyruvate dehydrogenase, which is a complex of 3 enzymes. The activity of one of the enzymes in this complex is suppressed by phosphorylation by pyruvate dehydrogenase kinase 1 (PDK1), which is a HIF-1 target. Therefore, through activation of PDK1, HIF-1 inhibits the conversion of pyruvate to Acetyl CoA. A potential downfall for glycolysis in this case would be the decrease in regeneration of NAD + , a necessary cofactor during glycolysis, as a result of the oxidative phosphorylation. However, the activity of lactate dehydrogenase, another target of HIF-1, regenerates NAD + , therefore contributing to the high activity of glycolysis detected in cancer cells.

HIF-1 induces the expression of miR-210, which targets the iron-sulfur cluster assembly protein 1/2, necessary for the activity of enzymes of the TCA as aconitase.

HIF-1 also promotes the PPP pathway through the induction of increased PKM2 expression, which promotes the accumulation of glycolysis intermediates, since the activity of this enzyme is weaker than other pyruvate kinase isoforms, therefore generating less pyruvate and accumulating glycolysis intermediates, mainly glucose-6-phosphate [18].

3.2 c-MYC

c-Myc is a helix-loop-helix type transcription factor that controls the expression of several genes related to cell growth. c-Myc dimerizes with Max to bind to the consensus element named E-box present in the promoter of several genes, which products are important for the regulation of protein biosynthesis, cell cycle control, metabolism, cell adhesion, and cytoskeleton modifications [18]. Its expression is triggered by mitogenic signals, through tyrosine kinase receptors, and signaling pathways as RAS/MAPK, PKC, PI3K/Akt and others. c-Myc is a proto-oncogene overexpressed in a high percentage of human cancers. To date, c-Myc has more than 10.000 target genes identified in the human genome. Due to its central function in cell growth and proliferation, c-Myc expression is controlled at all possible levels, from transcription to post-translational modifications that regulate its half-life. As HIF-1, c-Myc also targets genes that promote glycolysis. This includes GLUT-1, hexokinase, lactate dehydrogenase, glucose phosphate isomerase, glyceraldehyde 3-phosphate dehydrogenase, triosephosphate isomerase, phosphoglycerate kinase 1 and enolase. On the other hand, c-Myc also targets several genes which products mediate anabolic metabolism. This includes the multifunctional CAD protein (carbamoyl phosphate synthetase 2, aspartate transcarbomylase, and dihydroorotase), which is essential for the pyrimidine biosynthesis pathway; serine hydromethyltransferase, which catalyzes the conversion of serine in glycine, initiating the one-carbon metabolism; fatty acid synthase, an enzyme complex that regulates the de novo biosynthesis of long chain fatty acids as palmitate from acetyl and malonyl CoA, in the presence of NADPH; ornithine decarboxylase, the enzyme that catalyzes the first step of the pathways that leads to the synthesis polyamines, small aliphatic polycations essential to cell growth and proliferation, DNA stabilization, and the regulation of transcription and translation.

Interestingly, at the same time that c-Myc promotes glycolysis, it can directly inhibit peroxisome proliferator-activated receptor α (PGC1-α) suppressing mitochondria respiration. Moreover, as described for HIF-1, c-Myc also induce PDK1, blocking entry of pyruvate in the mitochondria. However, c-Myc can also activate mitochondria biogenesis, which would be necessary to ensure viability of daughter cells. Moreover, c-Myc upregulates the expression of glutamine transporters, increasing glutamine uptake. Together with its effect on the flux of 3-phosphoglycerate from glycolysis to serine synthesis, it is again clear the c-Myc role in anabolic pathways.

c-Myc can collaborate with HIF-1 to promote metabolic reprograming in cancer cells. Interestingly, in normal cells, HIF-1 inhibits c-Myc transcriptional activity by several mechanisms. For instance, HIF-1 binds to c-Myc and prevents its association with its transcriptional partner Max. Besides, HIF-1 activates c-Myc's antagonist Mxi-1, competes with c-Myc for consensus binding sites (5′-G/ACGTG-3′ vs. 5′-CACGTG-3′, respectively), and displace the transcription factor Sp1, which is essential for c-Myc promoter activation. However, these events do not occur in cancer cells, where c-Myc expression levels are elevated enough to counteract the effects described above [19].

3.3 PI3K/AKT/mTOR

Phosphatidylinositol 3-kinases (PI3K) is a proto-oncogene that plays an essential role in transmitting extracellular growth signals to the cell, promoting macromolecule biosynthesis, cell cycle progression and cell survival. In cases of gain of function, as is the case in many cancer cells, the PI3K pathway can promote cell growth in the absence of external mitogenic signals. PI3K phosphorylates phosphatidylinositol, a scarce phospholipid component of cell membrane generating second messengers for signaling such as inositol 1,4,5 triphosphate (IP3) and diacylglycerol. IP3, in turn, controls cytosolic Ca_2^+ levels while diacylglycerol activates the serine-threonine protein kinase C (PKC). In cancer, there is mostly the involvement of class one members of PI3Ks, from the 3 known classes. The class I members are activated by insulin and other growth factors and are divided in subgroups A and B, activated by tyrosine receptors and G protein-coupled receptors, respectively. PI3K is composed by two subunits, one with catalytic activity (p110α, β or δ) and one with regulatory activity (p85 α, β, γ p50α, p55α). The expression of these isoforms varies among tissues and cells, with exception of p110α that is ubiquitously expressed. Several types of human cancers display genetic alterations in PI3K members, from mutations to amplifications [12]. Some mutations in p85 prevent its inhibitory activity on p110 in the absence of mitogenic stimulus, leading to constitutive PI3K activation. One of the main targets of PI3K is the kinase Akt, recruited to the membrane by PIP3, generated once PI3K is activated. The recruitment of Akt to the membrane allows proximity to 3-phosphoinositide-dependent protein kinase (PDK1), which phosphorylates Akt, activating it. PI3K also activates the Mammalian target of rapamycin (mTOR) complex that also phosphorylates Akt contributing to its activation.

mTOR is a serine/threonine protein kinase that is part of two different protein complexes, mTORC1 and mTORC2. These complexes have different composition, are activated by different stimuli and activate different effector pathways. mTORC1 is activated by growth factors and nutrient availability and is composed of three core components: mTOR, Raptor (regulatory protein associated with mTOR) and mLST8 (mammalian lethal with Sec13 protein 8) and is inhibited by rapamycin. mTORC2 is composed also by mTOR, mLST8 and Rictor (rapamycin insensitive companion of mTOR), is activated by mitogenic stimuli and does not respond to acute rapamycin

treatment. One important activity of mTORC2 is activation of Akt, which has a central role in cell growth, proliferation and survival [20].

mTORC1 has a central role in the control of anabolism and catabolism in response to nutrient availability. As effector functions, mTORC1 phosphorylates the kinase S6K1 and eIF4E binding protein (4EPB), which leads to increase in protein synthesis. S6K1 activates substrates that promote initiation of mRNA translation, while phosphorylation of 4EBP releases eIF4E, allowing 5'cap-dependent mRNA translation to occur. mTORC1 promotes lipid synthesis through the sterol responsive element binding protein transcription factors (SREBP), both through S6K1 dependent and independent mechanisms. mTORC1 promotes nucleotide synthesis through activation of the folate cycle that provides one-carbon units for purine synthesis (Fig. 6). Finally, and expectedly, mTORC1 promotes glycolysis by increasing HIF-1 translation. Also, through activation of SREBP and regulation of glucose-6P-dehydrogenase, mTORC1 leads to increased flux of PPP [21]. mTORC1 is negatively regulated by the protein Tuberous Sclerosis Complex (TSC). TSC, in turn, is a target of Akt and Ras/Erk/$p90^{RSK}$. When phosphorylated by these complexes, TSC is inhibited, allowing mTORC1 activation. Another negative regulator of mTORC1 is the AMP activated protein kinase (AMPK). AMPK is an energy sensor activated by cellular AMP. AMP binds directly to this kinase causing an activating conformational change that leads to the phosphorylation of several targets, including TSC leading to its activation. Due to its role in the negative regulation of mTORC1, AMPK has been considered an antitumorigenic protein.

Akt is a key protein kinase that phosphorylates several downstream targets, among them proteins involved in glucose uptake, glycogen synthesis, cell growth and cell survival. Specifically, Akt phosphorylates HK, increasing glycolysis. Also, through phosphorylation and inhibition of glycogen synthase kinase 3 (GSK3), which inhibits both c-Myc and SREBP, Akt allows expression of these factors. Moreover, through activation of mTORC1, Akt also leads to activation of HIF-1, therefore contributing to the aerobic glycolysis increase. In fact, in cells with sustained Akt activity the glycolytic metabolism prevails over oxidative phosphorylation. Since these cells are dependent on glucose for survival this observation confirms the role of Akt in metabolic reprogramming. Besides, Akt also has a role in the translocation of GLUT4 to the plasma membrane. There is also experimental evidence showing that Akt can promote GLUT1, the preferentially expressed glucose transporter in cancer cells, translocation to the membrane [22, 23]. Interestingly, constitutive Akt activation leads to increase in GLUT1 mRNA and protein, which could be mediated by c-Myc.

Both Akt and S6K can phosphorylate phosphofructokinase 2 (PFK2) to increase its activity, which results in the generation of fructose 2,6-biphosphate. This is an important allosteric activator of phosphofructokinase 1 (PFK1), the enzyme that catalyzes one of the key reactions in glycolysis (Fig. 4). The PI3K/Akt signaling axis also activates glutaminolysis through c-Myc, which increases the expression of glutamine transporters. Besides, the reaction that converts glutamine to glutamate is catalyzed by glutaminase (GLS). The reverse reaction is catalyzed by glutamine synthetase (GLUL), which condenses glutamate and ammonia in an ATP-dependent reaction to form glutamine. Both enzymes can be regulated by PI3K/Akt/mTOR

pathway: GLS is a c-Myc target gene and GLUL expression, which is increased by FoxO, is not expressed upon FoxO inactivation by Akt [24].

Besides contributing to the glycolytic metabolism, the PI3K/Akt pathway also contributes to anabolic metabolism. First through activation of mTORC1, resulting in the effects described previously in this chapter, but also through phosphorylation of ATL citrate lyase, which converts citrate to cytosolic Acetyl CoA, required for lipid biosynthesis. At the same time, Akt inhibits FoxO transcription factor, which suppresses lipogenesis when active, by downregulation of lipogenic genes expression.

An important regulator of this pathway is PTEN, a *bona fide* tumor suppressor, which removes the 3'phosphate of PIP3, shutting down downstream signaling. Loss of PTEN function, therefore, prolongs the half-life of the PI3K/Akt signaling and is associated with several types of cancer in humans.

3.4 Tp53

TP53 is probably the most famous tumor suppressor gene. Its product is a transcription factor that binds as a tetramer to the promoter regions of genes involved in apoptosis and DNA damage responses, protecting tissues from cells harboring oncogenic mutations. TP53 mutations can result in loss of function or altered function and protein stability. However, TP53 has also a role as a stress monitor: perturbations in oxygen and nutrient availability can induce TP53 expression either by protein stabilization or post-translational modifications. Therefore, in response to metabolic stress, TP53 can transactivate stress-response genes. For instance, activation of AMPK, previously discussed in this chapter can activate TP53, linking high AMP/ATP ratio to TP53 activity. Moreover, nutrient depletion can inactivate mTORC1. mTORC1 inhibits TP53, therefore, in this situation TP53 can perform transactivation activity. When nutrient availability increases, mTORC1 is activated and rapidly inhibits TP53. In concert with mTORC1 activity, Akt activates the ubiquitin ligase MDM2, which targets TP53 for proteasomal degradation. Again, the inactivity of Akt due to low nutrient availability may stabilize TP53. Interestingly, TP53 increases the expression of PTEN, which inhibits the PI3K pathway.

Mutations causing loss of function of TP53 are a very common in human cancers. Somatic mutations in this gene occur in almost every cancer type in humans, with rates varying from 30 to 50% in solid cancers as ovarian, esophageal, colorectal, head and neck, larynx and lung carcinomas. In cancers that do not display TP53 mutations, there are usually inactivation mechanisms. For example, in Human Papillomavirus associated cancers, one of the viral oncoproteins targets TP53 for proteasomal degradation [25]. Loss of TP53 function implicates in loss of its regulatory mechanisms over metabolism, which contributes to the metabolic reprogramming.

TP53 activity counteracts mTORC1 by decreasing glycolysis rate and increasing oxidative phosphorylation. That is accomplished by inhibition of GLUT1, GLUT4

and phosphoglycerate expression, and by increasing the expression of TP53-induced glycolysis and apoptosis regulator (TIGAR), an enzyme that reduces the levels of fructose-2,6-biphosphate, an allosteric activator of phosphofructokinase. TP53 also limits the expression of monocarboxylate transporters (MCT), responsible for lactate and other monocarboxylates traffic through the plasma membrane. Moreover, TP53 negatively regulates HIF-1, through competition for enhancer binding in mild hypoxia and by promoting HIF-1 proteasome degradation under severe hypoxia, and c-Myc, which transcription is directly repressed by TP53, abrogating their effect on metabolic reprogramming [26].

On the other hand, TP53 upregulates the synthesis of cytochrome c oxidase gene, one of the electron transporters of oxidative phosphorylation. TP53 also induces transcription of SCO2, a copper transporter that is critical for the assembly of cytochrome oxidase. Moreover, TP53 promotes mitochondrial integrity through expression of mitochondria-eating protein (MIEAP), which regulates mitophagy, a process important for mitochondria quality and turnover. Besides, TP53 promotes increase TCA turnover through expression of glutaminase 2 (GLS2), a mitochondrial enzyme that catalyzes conversion of glutamine to glutamate (Fig. 5), which is then converted to α-KG feeding the TCA. Active TP53 can also inhibit the malic enzyme, decreasing the conversion of malate to pyruvate, and trapping malate in the mitochondria (Fig. 5).

Finally, TP53 also promotes β-oxidation while decreasing fatty acids synthesis. The mechanisms behind these processes include the induction of carnitine palmitoyl transferase 1C (CPT1C), promoting the import of fatty acids to the mitochondria, where β-oxidation occurs. It also induces the expression of acyl-CoA dehydrogenase that catalyzes the initial step of β-oxidation. On the other hand, TP53 suppresses SREBP1, a regulator of fatty acids synthesis, both through direct transcriptional repression and by increasing the expression levels of its inhibitor LIPIN1.

Hypoxia is an important regulator of TP53 in a complex and gradient dependent manner. Chronic hypoxia seems to lead to accumulation of inactive TP53 (lack of post translational modifications necessary for transactivation activity), mediated by HIF-1, which binds to enhancer sequences in the TP53 promoter region. On the other hand, severe hypoxia or anoxia leads to activation of TP53, resulting in induction of apoptosis. There is experimental evidence that, during chronic hypoxia transcriptionally inactive TP53, either wild type or mutated, binds to HIF-1, serving as a molecular chaperone to HIF-1. This interaction stabilizes HIF-1 binding to its responsive elements in gene promoters and affecting gene expression [9].

4 Effects of Tumor Cell Metabolism on Cancer Progression

It is clear by now that cancer cells uptake nutrients from the microenvironment, promote its acidification, and release lactate. This cancer conditioned microenvironment has many effects on cancer cells themselves as well as in stromal cells.

Lactate, a byproduct of glycolysis has a central role in interactions among tumor cells and between tumor cells and stromal ones. A process known as metabolic symbiosis can occur between glycolytic tumor cells in regions with lower oxygen availability and oxidative tumor cells in regions with higher oxygen availably, near blood vessels. There is published data showing differential expression of MCT family members in these cell populations allowing for lactate secreted by glycolytic cells to be consumed as a substrate to oxidative phosphorylation by oxidative cells. This process would be mediated, at least in part, by HIF-1, which promotes expression of MCT4. MCT4 has low lactate affinity for lactate compared to MCT-1 and therefore, in cancer cells, where there is high lactate concentration, this transporter can export lactate. On the other hand, MCT1, expressed in cells close to blood vessels, can uptake lactate due to its high affinity to this metabolite (<2 mM), allowing its use as a nutrient [27]. Likewise, this cycle is also realized between cancer cells and glycolytic cancer-associated fibroblasts (CAFs), which can also secrete lactate and pyruvate, which are then used through the TCA in cancer cells, in the so-called reverse Warburg effect [28].

Metastasis, another hallmark of cancer cells, is the colonization of tissues adjacent or distant to the primary tumor. Metastatic cancer cells gain the ability to migrate and invade other tissues, eventually proliferating in these tissues and generating secondary tumors. The main byproducts of glycolysis are the production of lactate and acidification of the extracellular pH. Both pH acidification as lactate can activate NFKB signaling in tumor cells, leading to secretion and activation of hydrolases as cathepsins and matrix metallopeptidases, which are important for tissue invasion. Activation of NFB has also been associated with epithelial mesenchymal transition, a cardinal feature of invasive carcinomas. Once cells detach of the basal membrane to migrate or even circulate, tumor cells seem to rely on antioxidant metabolism to survive. In this case, the flux of PPP to generate NAPDH is important for matrix detached cell survival. As mentioned before, by reducing glutathione, NADPH is key in maintain redox balance in cancer cells. Finally, exosomes released by tumor cells contain miR-122, which suppresses glucose metabolism in resident cells in pre-metastatic niches, by downregulating PKM2 and GLUT1, leading to availably of glucose for metastatic seeding [29].

Besides metastasis, there is evidence that cancer cell metabolism is linked to chemo and radiotherapy resistance. For example, in cervical cancer, lactate can also reinforce DNA repair and promote resistance to chemotherapy, through inactivation of histone deacetylase. Moreover, synthesis of nucleotides in cancer cells is essential for DNA repair after extensive mutations caused by treatment. Finally, as mentioned above, NADPH role in regulating redox balance is essential in cells treated with chemo or radiotherapy, both of which induce oxidative stress.

Lactate and acidic pH can also mediate angiogenesis. Lactate, uptaken by endothelial cells through MCT1, activates NFκB and its target IL-8, driving endothelial cell migration and tube formation. Likewise, it activates HIF-1, even in normoxia, promoting angiogenesis, through activation of VEGFR2 and basic FGF, both growth factors necessary of endothelial cell proliferation. Finally, acidosis, in a hypoxia independent manner, modulates VEGF expression. Low pH leads to increase in VEGF

concentration at both mRNA and protein levels. Moreover, acidic pH regulates VEGF splicing favoring the angiogenic VEGF-121 [30]. Other than that, lactate, acidic pH and the competition for nutrients also have important effects over immune cells that infiltrate the tumor microenvironment, as discussed next.

Immune responses are important for controlling and eliminating tumors. Immune escape is one of the cancer hallmarks [1]. Several immune escape mechanisms have been described. Nowadays, it is clear that metabolites and microenvironment conditions caused by cancer cell metabolism also have a role in suppression of anti-tumor immune responses. Depletion of nutrients in the tumor microenvironment, can restrict T cell responses through competition for glucose and amino acids. Effector T cells have a metabolic profile similar to cancer cells, relying on glycolysis for activation and Interferon secretion, an important effector molecule for cytotoxic immune responses [31]. Other than that, macrophages with M1-like phenotype, activated dendritic cells and NK cells also rely on glycolysis to exert its antitumor activities. As tumor cells upregulate molecules that increase the uptake of nutrients from the environment, other cells are then exposed to lower nutrient concentration. Moreover, the immune cells modulated by the tumor microenvironment towards an immunosuppressive phenotype, as macrophages with M2-like phenotype, myeloid-derived suppressor cells (MDSC) and tolerogenic dendritic cells contribute to the decreased availability of nutrients, also consuming, for example, glucose, arginine, cysteine and tryptophan.

Besides the competition for nutrients, the tumor microenvironment metabolism also contributes to the impairment of antitumor responses through the products generated with the metabolic activity of the tumor and associated cells. For example, glutamine consumption leads to accumulation of glutamate, which suppresses T cell activity [32]. Tumor cells and antigen presenting cells in tumors, express an enzyme, indoleamine 2,3-deoxygenase, that catalyzes the conversion of tryptophan to kynurenines. The last ones capable of inducing regulatory T cells differentiation [33], suppressing therefore anti-tumor immune responses. Finally, a major metabolite regulating immune responses in the tumor microenvironment is lactate. Lactate inhibits cytotoxic cells as NK cells and CD8 T cells, it can promote M2-like phenotype on macrophages, and can induce tolerogenic phenotype on dendritic cells that, in turn, inhibit T cell responses or induce the differentiation of regulatory T cells. Lactate can signal to other cells either entering cells through MCTs, or by binding to G-protein coupled receptors, mainly GPR81, which increases the levels of cAMP in the cell and activates PKA (cAMP dependent Protein Kinase). Activation of GPR81 promotes regulation of immune responses through suppression of inflammatory cytokines expression [34, 35]. Lactate can also induce the expression of PD-L1 in tumor cells [36]. PD-L1 is a negative regulator of T cell responses, inducing the exhaustion phenotype. PD-L1, through Akt, increases glycolytic metabolism in tumor cells [31], leading to increase in glucose uptake and lactate secretion, enhancing the immunosuppressive environment.

5 Final Remarks

As expected, as researchers better understand cancer cell metabolic reprograming and its effects on the tumor microenvironment and biology, several potential therapeutic tools have been suggested. Therapeutic possibilities targeting cancer cell metabolism are several: treatment with bicarbonate or other buffers to increase tumor pH; inhibition of glutamine metabolism either by inhibition of glutaminase (the enzyme that catalyzes the conversion of glutamine into glutamate) or by blocking the transporter alanine-serine-cysteine transporter 2 (the major glutamine transporter in cancer cells); inhibition of lipids synthesis through inhibition of fatty acids synthase (FASN) a multienzyme important in the final process of FA synthesis, or acetyl-CoA carboxylase inhibition, the enzyme that catabolizes the conversion of acetyl-CoA to malonyl-CoA, a rate limiting reaction in FA synthesis; impairment of pyruvate dehydrogenase and α-KG dehydrogenase by high lipoate analog concentration can disrupt the TCA; blockade of MTCs, mainly MCT1 has been tested in clinical trials. Actually, several of these options have been tested or are in test in clinical trials [37]. Although the effects of such treatment should be taken into consideration, since antitumor leukocyte populations usually have similar metabolic profiles as tumor cells, the manipulation of cancer cell metabolism is clearly a promising area to be explored to treat cancer.

References

1. Hanahan D, Weinberg RA (2011) Hallmarks of Cancer: The Next Generation. Cell 144:646–674
2. Garcia-Heredia JM, Carnero A (2015) Decoding Warburg's hypothesis: tumor related mutations in the mitochondrial respiratory chain. Oncotarget 6:41582–41599
3. Hsu PP, Sabatini DM (2008) Cancer cell metabolism: Warburg and beyond. Cell 134:703–707
4. Israelsen WJ, Dayton TL, Davidson SM, Fiske BP, Hosios AM, Bellinger G et al (2013) PKM2 Isoform-specific deletion reveals a differential requirement for pyruvate kinase in tumor cells. Cell 155:397–409
5. Zhang B, Pan X, Cobb GP, Anderson TA (2007) MicroRNAs as oncogenes and tumor suppressors. Dev Biol 302:1–12
6. Duronio RJ, Xiong Y. Signaling pathways that control cell proliferation. Cold Spring Harbor Perspect Biol. 2013, 5: a008904.
7. Kroemer G, Pouyssegur J (2008) Tumor cell metabolism: Cancer's Achilles Heel. Cancer Cell Rev. 13:472–482
8. Lis P, Dylag M, Niedzwiecka K, Ko YH, Pedersen PL, Goffeau A, Ulaszewski S (2016) The HK2 dependent "Warburg Effect"and Mitochondrial Oxidative Phosphorylation in Cancer: Targets for Effective Therapy with 3-Bromopyruvate. Molecules 21:1730
9. Madan E, Parker TM, Pelham CJ, Palma AM, Peixoto ML, Nagane M et al (2019) HIF-transcribed p53 chaperones HIF-1α. Nucl Acids Res. 47:10212–10234
10. Yang L, Venneti S, Nagrath D (2017) Glutaminolysis: a hallmark of cancer metabolism. Annu Rev Biomed Eng 19:163–194
11. Jose C, Bellance N, Rossignol R. Choosing between glycolysis and oxidative phosphorylation: a tumor's dilemma? Bioch Biophys Acta—Bioenerg. 2011, 1807: 552–561.

12. Koizume S, Miyagi Y (2016) Lipid droplets: a key cellular organelle associated with cancer cell survival under normoxia and hypoxia. Int J Mol Sci 17:1430
13. Carracedo A, Cantley LW, Pandolfi PP. Cancer metabolism: fatty acid oxidation in the limelight. Nat Rev Cancer. 2013, 13: 227 - 232.
14. Koundouros N, Poulogiannis G (2019) Reprogramming of fatty acid metabolism in cancer. British J Cancer. 122:4–22
15. Selvarajah B, Azuelos I, Platé M, Guillotin D, Forty EJ, Contento G et al (2019) mTORC1 amplifies the ATF4-dependent de novo serine-glycine pathway to supply glycine during TGF-α1-induced collagen biosynthesis. Sci Sig. 12:3048
16. Mattaini KR, Sullivan MR, Vander Heiden. The importance of serine metabolism in cancer. J Cell Biol. 2016, 213: 249—257.
17. Smolková K, Bellance N, Scandurra F, Génot E, Gnaiger E, Plecitá-Hlavatá L et al (2010) Mitochondrial bioenergetic adaptations of breast cells to aglycemia and hypoxia. J Bioenerg Biomemb 24:55–67
18. Dang CV, O'Donell KA, Zeller KI, Nguyen T, Osthus RC, Li F (2006) The c-Myc target gene network. Semin Cancer Biol 16:253–264
19. Soga T (2013) Cancer metabolism: key players in metabolic reprogramming. Cancer Sci 104:275–281
20. Saxton RA, Sabatini DM (2017) mTOR Signaling in Growth, Metabolism, and Disease. Cell 168:960–976
21. Tsouko E, Khan Ma, White MA, Han JJ, Shi Y, Merchant FA, et al. Regulation of the pentose phosphate pathway by an androgen receptor-mTOR-mediated mechanism and its role in prostate cancer cell growth. Oncogenesis. 2014, 3: e103.
22. Bentley J, Itchayanan D, Barnes K, McIntosh E, Tang X, Downes CP et al (2003) Interleukin-3-mediated cell survival signals include phosphatidylinositol 3-kinase-dependent translocation of glucose transporter GLUT1 to the cell surface. L Biol Chem 278:39337–39348
23. Rathmell JC, Fox CJ, Plas DR, Hammerman PS, Cinalli RM, Thompson CB (2003) Akt-directed glucose metabolism can prevent Bax conformation change and promote growth factor-independent survival. Mol Cell Biol 23:7315–7328
24. Bott AJ, Peng IC, Fan Y, Faubert B, Zhao L, Li J et al (2015) Oncogenic Myc induces expression of glutamine synthetase through promoter demethylation. Cell Metab 22:1068–1077
25. Olivier M, Hollstein M, Hainaut P. TP53 mutations in human cancers: origins, consequences, and clinical uses. Cold Spring Harbor Perspect Biol. 2010, 2: a001008.
26. Gomes AS, Ramos H, Soares J, Saraiva L (2018) p53 and glucose metabolism: an orchestra to be directed in cancer therapy. Pharmacol Res 131:75–86
27. De la Cruz-López KG, Castro-Muñoz LJ, Reyes-Hernández DO, García-Carrancá A, Manzo-Merino J (2019) Lactate in the regulation of tumor microenvironment and therapeutic approaches. Frontiers Oncol. 9:1143
28. Pavlides S, Whitaker-Menezes D, Castello-Cros R, Flomenberg N, Witkiewicz AK, Frank PG et al (2009) The Reverse Warburg Effect: Aerobic Glycolysis in the Cancer Associated Fibroblasts and the Tumor Stroma. Cell Cycle 8:3984–4001
29. Elia I, Doglioni G, Fendt SM (2018) Metabolic hallmarks of metastasis. Trends Cell Biol 28:673–684
30. Thews O, Riemann A (2019) Tumor pH and metastasis: a malignant process beyond hypoxia. Cancer Met Rev. 38:113–129
31. Chang C, Qiu J, O'Sullivan D, Buck MD, Noguchi T, Curtis JD et al (2015) Metabolic Competition in the Tumor Microenvironment is a Driver of Cancer Progression. Cell 162:1229–1241
32. Pacheco R, Gallart T, Lluis C, Franco R (2007) Role of glutamate on T-cell mediated immunity. J Neuroimmunol 185:9–19
33. Fallarino F, Grohmann U, You S, McGrath BC, Cavener DR, Vacca C et al (2006) The combined effects of tryptophan starvation and tryptophan catabolites down-regulate T cell receptor zeta-chain and induce a regulatory phenotype in naive T cells. J Immunol 176:6752–6761

34. Baltazar R, Afonso J, Costa M, Granka S (2020) Lactate beyond a waste metabolite: metabolic affairs and signaling in malignancy. Front Oncol 10:231
35. Ranganathan P, Shanmugam A, Swafford D, Suryawanshi A, Bhattacharjee P, Hussein MS et al (2018) GPR81, a cell-surface receptor for lactate, regulates intestinal homeostasis and protects mice from experimental colitis. J Immunol 200:1781–1789
36. Feng J, Yang H, Zhang Y, Wei H, Zhu Z, Zhu B et al (2017) Tumor cell-derived lactate induces TAZ-dependent upregulation of PD-L1 through GPR81 in human lung cancer cells. Oncogene 36:5829–5839
37. Sun NY, Yang MH (2020) Metabolic Reprogramming and Epithelial-Mesenchymal Plasticity: Opportunities and Challenges for Cancer Therapy. Front Oncol 10:792
38. Anderson NM, Mucka P, Kern JG, Feng H (2018) The emerging role and targetability of the TCA cycle in cancer metabolism. Protein Cell 9:216–237
39. Gonçalves MD, Hopkins BD, Cantley LW (2018) Phosphatidylinositol 3-kianse, growth disorders, and cancer. NEJM 379:2052–2062
40. Hunter T (1997) Oncoprotein networks. Cell 88:333–346
41. Mathupala SP, Ko YH, Pedersen PL (2006) Hexokinase II: Cancer's double-edged sword acting as both facilitator and gatekeeper of malignancy when bound to mitochondria. Oncogene 25:4777–4786
42. Nagao A, Kobayashi M, Koyasu S, Chow CCT, Haranda H (2019) HIF-1-dependent reprogramming of glucose metabolic pathway of cancer cells and its therapeutic significance. Int J Mol Sci 20:238–251
43. Patra KC, Hay N (2014) The pentose phosphate pathway and cancer. Trends Biochem Sci 39:347–354
44. Stincone A, Prigione A, Cramer T, Wamelink MMC, Campbell K, Cheung E et al (2015) The return of metabolism: biochemistry and physiology of the pentose phosphate pathway. Biol Rev 90:927–963
45. Warburg O (1956) On the origin of cancer cells. Science 123:309–314

Adipose Tissue and Immuno-Metabolic Regulation

Gustavo Gastão Davanzo, Alexandria Hoffman, Pedro Manoel Mendes de Moraes-Vieira, and Lev Becker

Abbreviations

AT	Adipose tissue
BAT	Brown adipose tissue
FA	Fatty acids IL-6
Interleukin 6 IRS-1	Insulin receptor substrate-1
SVF	Stromovascular fraction T2D
Type 2 diabetes Treg	Regulatory T cells
TNF-α	Tumor necrosis factor alpha
UCP-1	Uncoupling protein-1
VLDL	Very low-density lipoproteins WAT
WAT	White adipose tissue

Gustavo Gastão Davanzo and Alexandria Hoffman Contributed equally.

G. G. Davanzo · A. Hoffman · L. Becker (✉)
Committee On Molecular Metabolism and Nutrition, The University of Chicago, Chicago, IL, USA
e-mail: levb@uchicago.edu

Ben May Department for Cancer Research, The University of Chicago, Chicago, IL, USA

G. G. Davanzo · P. M. M. de Moraes-Vieira (✉)
Laboratory of Immunometabolism, Department of Genetics, Evolution, Microbiology and Immunology, Institute of Biology, University of Campinas, Campinas, Brazil
e-mail: pmvieira@unicamp.br

N. O. S. Camara et al. (eds.), *Essential Aspects of Immunometabolism in Health and Disease*, https://doi.org/10.1007/978-3-030-86684-6_10

1 Introduction

Adipose is a dynamic tissue that is uniquely able to store and mobilize lipid. An essential function of adipose tissue is to regulate whole body energy homeostasis; this function is the domain of white adipose tissue. A second type of adipose tissue, referred to as brown fat, mobilizes lipid to dissipate its chemical energy in the form of heat. Furthermore, several other functions of adipocytes have been described and the adipose tissue as a whole contains numerous other cell types. These non-adipocyte cells are referred to as the stromovascular fraction (SVF) and include stem cells, immune cells, and vascular cells. The SVF can influence adipocytes and be influenced by adipocytes in return. In this chapter we will discuss the two principal types of adipose tissue (AT): white (WAT) and brown (BAT) and the relationship between adipocytes and immune cells in these environments.

Across evolutionary phylogeny the storage of energy as lipids is well conserved. While prokaryotes and single-cell eukaryotes store lipids in intracellular organelles known as lipid droplets, multicellular organisms developed specialized cells to store them, called adipocytes [1]. In humans lipid enters the circulation via two major pathways: (1) dietary uptake of fatty acids (FAs) that are packaged as chylomicrons and (2) triglycerides produced by the liver that are delivered into the blood as very low-density lipoproteins (VLDL) in the circulation.

Dietary FAs are absorbed in intestinal enterocytes where they are secreted as chylomicrons: a core of triglycerides surrounded by a monolayer membrane of phospholipids, and apolipoproteins. Chylomicrons are then delivered into the circulation. The liver can package its stored lipid into VLDL, a lipoprotein with a core of triglycerides and some cholesterol covered by a phospholipid and apolipoprotein coat. VLDL is then released into the circulation for transport to peripheral tissue.

2 Lipogenesis

Once in the circulation, chylomicrons and VLDL cannot cross the endothelium of blood vessels. To absorb the lipid cargo, adipocytes synthesize the enzyme lipoprotein lipase (LPL) and secrete it into the capillary where it can hydrolyze triglycerides into smaller FAs and 2-monoacylglycerol. These FAs enter adipocytes by passive diffusion or by active uptake through receptor proteins, including FA transport proteins, FA translocase (CD36) and plasma membrane FA binding protein. When inside the adipocytes, FAs and monoacylglycerol will be transformed to acyl-CoA, which is a key molecule in FA synthesis. Three molecules of acyl-CoA are esterified to one molecule of glycerol-3-phosphate (G-3-P), forming a triglyceride (G-3-P could be formed by glycolysis or glyceroneogenesis, both pathways could be reviewed in several textbooks and reviews as [2]). At the end of lipogenesis, triglycerides and cholesterol ester are stored in a large lipid droplet surrounded by a phospholipid monolayer and coated with many proteins. Through this process, adipose tissue

provides a temporary storage space for circulating lipids so they may be used later by the organism.

3 White Adipose Tissue (WAT)

As a major reserve of energy, adipocytes are a major player in the control of whole-body energy homeostasis. After feeding, when energy is plentiful, increased levels of glucose induce secretion of insulin by beta-cells in the pancreas. Insulin induces adipocytes to take up circulating glucose and FAs. This uptake is accompanied by activation of gene networks that induce more esterification of FAs and lipogenesis [3]. When food is scarce, glucagon (from alpha cells in the pancreas), glucocorticoids and catecholamines (from sympathetic nervous system) instructs adipocytes to hydrolyze lipid droplet triglycerides into FAs and glycerol. FAs enter the circulation and can be used by other cells in beta-oxidation and ATP generation. Furthermore, glycerol can be used for hepatic gluconeogenesis [4, 5]. Through the coordinated actions of hormones white adipose tissue acts as a storage depot for calories when energy is plentiful and a source of calories when it is scarce.

4 Brown Adipose Tissue (BAT)

Exclusively founded in mammals, BAT is named from the increased number of iron-rich mitochondria that give the tissue a brown color. Evolutionarily, researchers believe that BAT occurred very early in the evolution of mammals, through the acquisition of a new protein, uncoupling protein-1 (UCP-1). This protein is located within the mitochondria of brown adipocytes. The electrochemical gradient across the inner mitochondrial membrane is usually coupled to ATP synthesis via complex V of the electron transport chain. However, UCP-1 acts as a proton leak that allows protons to flow back to the mitochondrial matrix, leading to increased oxidation of glucose and lipids, and heat generation [6]. This pathway evolved to provide mammals with extra heat in diverse situations including postnatally, hibernation, febrile state or in a state of cold stress [7].

The genesis and activity of BAT is controlled by the central nervous system. The hypothalamus receives a spectrum of signals from the periphery, as changes in body temperature, feeding, and stress activate hypothalamic neurons which project fibers to the sympathetic nervous system. The sympathetic nervous system produces norepinephrine which activates B-adrenergic receptors on adipocytes. This causes many important changes in adipocytes including [1] lipolysis of white and brown adipocytes and (2) expression of UCP-1 (2) biogenesis of mitochondria and (4) lipid uptake by BAT [8].

BAT is primarily found in two depots: the interscapular and perirenal regions. Although the mass of BAT is much smaller than WAT in adults, newborns have a

higher percentage of BAT, presumably to protect small newborns against the cold after birth. However, recent discoveries showed that browning can be induced in white adipocytes, allowing them to take on a thermogenic capacity. The formation of these beige adipocytes is often induced by exposure to cold or β-adrenergic agonists [9–11]. The description of inducible browning provided a new avenue in the treatment of obesity that several groups are pursuing. Although the transplant of human beige adipocytes into obese mice leads to improvement in glucose homeostasis and reduced hepatic steatosis [12], more research is needed for brown adipose to become a feasible treatment for obesity in humans.

5 White Adipose Tissue and Obesity

The last 40 years have been marked by a worldwide obesity epidemic. Obesity, or a high body fat percentage, is associated with elevated levels of inflammation in many organs, including adipose tissue. Adipose tissue is responsible for storing excess nutrition and expands during obesity. Inflammation can interfere with the adipose tissue's ability to respond to the hormone insulin, which promotes the uptake of nutrients from the blood into the adipose. The decreased response to insulin, termed "insulin resistance", leads to increased risk for developing type 2 diabetes (T2D) and heart disease. In addition to its primary functions during obesity, the biology of adipose tissue plays other important roles in the development of obesity related diseases.

Earlier in this chapter we described the classical model of white adipose tissue (WAT) as a storage organ for excess nutrition to be mobilized during energy scarcity. However, it is now clear that this model is incomplete. In the last 30 years, adipose tissue has emerged as a major endocrine organ. Anatomically, WAT comprises both subcutaneous and visceral depots which are directly beneath the skin and surrounding internal organs, respectively. Visceral adipose is subdivided in mesenteric, omental, perirenal and peritoneal depots. In the section we will describe the role of adipose tissue as a source of hormone signals that orchestrate whole body energy regulation.

6 Adipocytes as Endocrine Cells

For a long time, adipocytes were only associated with the storage of lipids, however, in the last forty years researchers found that adipocytes can secreted hormones including: *adipokines* (leptin, adiponectin, resistin, chemerin, apelin, visfatin, plasminogen activator inhibitor 1.

(PAI1), retinol binding protein 4 (RBP4) monocyte chemoattractant protein 1 (MCP1) and *cytokines* (interleukin 6 (IL-6) and tumor necrosis factor alpha (TNF-α)) [1, 13]. Consequently, it is well accepted that adipose tissue is an endocrine organ

that regulates systemic metabolism and inflammation. In this topic, we will focus in the two well-characterized adipokines secreted by WAT: leptin and adiponectin.

7 Leptin

Leptin was the first characterized adipokine in the 1990s by Jeffrey Friedman's group as the hormone responsible for the control of food intake in the brain. Adipocytes are the main producers of leptin, however, other organs such as the stomach, intestine and muscle can also produce leptin [5]. Leptin is a small peptide (16 kDa) that is secreted into blood after feeding. It crosses the blood–brain barrier in the brain and acts mainly in the hypothalamus to cause satiety [5]. The effects of leptin are clearly demonstrated when using mice deficient in leptin expression (*ob*/*ob*) or receptor function (*db*/*db*), in both cases mice present with increased food intake and severe obesity [14, 15]. Interestingly, obesity is associated with increased levels of leptin due to hypothalamic leptin resistance [16]. In addition to its well stablished role In the brain, leptin can also activates many types of immune cells including dendritic cells, monocytes, macrophages, neutrophils, natural killer cells, and T cells. In this context, leptin is considered a proinflammatory adipokine that increases immune cell migration and accumulation in adipose tissue.

8 Adiponectin

A second well characterized adipokine is adiponectin, a 30 kDa peptide secreted by adipocytes. Adiponectin acts on adiponectin receptors 1 and 2 (AdipoR1 and AdipoR2) which are widespread throughout the body. When adiponectin binds its receptor in peripheral tissue it promotes insulin sensitivity. Furthermore, adiponectin can act in the hypothalamus to stimulate food intake [17]. Interest in adiponectin increased in the beginning of the twenty-first century, when several groups described that obese and type 2 diabetic patients have decreased levels of adiponectin in the circulation. It was hoped that adiponectin could be used as a biomarker and/or a target to treat the metabolic syndrome [18]. Indeed, one class of insulin-sensitizing drugs called thiazolidinediones (TZDs) work in part through increasing adiponectin secretion [19].

9 Retinol-Binding Protein 4 (RBP4)

RBP4 is a 21 kDa protein, secreted into the circulation by adipocytes and liver, where it is responsible for the retinol (vitamin A) transporter in blood. First studied in the context of ophthalmology, clinical trials described increased levels in obese and

insulin-resistant humans and mouse models. RBP4 activate macrophages, leading to proinflammatory cytokines production and activation of CD4 Th1 cells in the adipose tissue, which contribute to more inflammation.

9.1 Adipose Tissue Immune Cells

Adipose tissue is a complex environment composed of adipocytes and the immune, endothelial, and stem cells that support them. Once thought to be inert storage devices, adipocytes are now appreciated as integral sources of hormones that influence other cells in adipose tissue as well as orchestrate whole-body energy status. In the second half of this chapter we will investigate the immune cells that make up the adipose tissue microenvironment and their effect on the metabolic syndrome with an emphasis on insulin resistance.

There are multiple immune cell types, both innate and adaptive, that reside in adipose tissue and they have all been shown to affect the progression of the metabolic syndrome. However, one of the most studied of these cells is the adipose tissue macrophage. Because of its well-supported role in the development of the metabolic syndrome, we will pay particular focus to this cell type.

9.2 Macrophages

Macrophages are an incredibly dynamic cell of the innate immune system. Their discovery is credited to Elia Metchnikoff, who observed both their ability to extravasate through blood vessels and phagocytose microbes [20]. Macrophages originate primarily from the bone marrow as monocytes, however there are different populations of tissue resident macrophages that are seeded during embryonic development [21]. Bone-marrow derived macrophages will circulate as monocytes until they observe a signal of stress in peripheral tissue, which usually manifests as chemokines and adhesion molecules that allow the monocytes to adhere to the endothelial wall before migrating into the peripheral tissue [22]. Once the monocytes move from the blood into the peripheral tissue, they become macrophages and can respond to the stimuli in the environment. These stimuli cause macrophages to adopt a radical new phenotype, a process called polarization, depending on the nature of the stimulus. Polarization can be broadly split into two types: 1) pro-inflammatory classical polarization for pathogen destruction and 2) anti-inflammatory alternative activation for tissue repair.

Our understanding of macrophages in the context of infection is very thorough because it is where they were originally discovered and studied. The innate immune system, and macrophages in particular, act as a jack-of-all trades to protect the host against a wide range of pathogens and insults. This requires the macrophage to respond to a staggering variety of signals. Two broad categories of signals

are pathogen associated molecular patterns (PAMPs), indicating the presence of a microbe, and danger associated molecular pattern (DAMPs), which indicate the presence of cellular damage and stress. When they encounter signs of a pathogen macrophages can destroy them using multiple strategies including [1] sequestration of resources (2) release of cytotoxic materials and (3) direct phagocytosis and destruction of the pathogen. Macrophages can also respond to signals from the adaptive immune system in the form of antibodies and cytokines. Of primary importance in the interaction between macrophages and the adaptive immune system is their role as antigen presenting cells (APCs). Macrophages can internally process a pathogen and present a piece of it to the adaptive immune system as a kind of "wanted poster" for invading microbes. By recognizing PAMPs and DAMPs, directly killing pathogens, and alerting the adaptive immune system, macrophages are able to act as a powerful sentinel for host defense.

Recently, investigators have understood that macrophages play many more roles than host defense. Resident macrophages that make their niche in peripheral tissue have a much more long-term homeostatic role. Here they perform maintenance jobs such as identifying and clearing dying cells, promoting blood vessel growth, and remodeling the extracellular matrix [23]. Adipose tissue macrophages play such a role in both a lean and obese state. In 2003 it was found that macrophages accumulate in adipose tissue during obesity and that they are a major source of the inflammation that contributes to insulin resistance [24]. In the following sections we will explore the nature of adipose tissue macrophages during obesity and its effect on insulin sensitivity.

9.3 Adipose Tissue Macrophages

Macrophages were first studied in the context of infection which gave rise to the paradigm of classical (M1) and alternative (M2) activation. As investigators studied tissue macrophages outside of infection they attempted to place them in either of these two categories. However, it was noted that the M1 and M2 designations were not able to explain the complexity of macrophage phenotypes observed during obesity. For example, adipose tissue macrophages during obesity present a dilemma because they have characteristics of both classical and alternative activation. In this section we will discuss the evidence for both classical and alternative activation in adipose tissue macrophages and their role during obesity.

Classical activation is characterized by the coordinated movement of macrophages to a site of stress followed by inflammation and/or tissue damage. This framework holds true during obesity. As adipose tissue expands during weight gain the individual adipocytes become inflamed. This inflammation can (1) activate the nearby macrophages and (2) attract more macrophages to the area [23]. These activated macrophages produce inflammatory cytokines such as TNF-α, IL-6, and interleukin 1 beta (IL-1β) which are characteristic of classical activation. Studies of adipose tissue macrophages before and after obesity demonstrate that they become inflamed

after weight gain. The inflammation produced by macrophages impacts insulin sensitivity in adipocytes. We know that macrophage inflammation is a critical step in this process because depleting macrophages or inhibiting their inflammation protects mice from insulin resistance [25–27]. A critical distinction between infection and obesity is the severity of inflammation. While they share common cytokines the levels of these inflammatory cytokines are almost 20 times higher in infection than obesity.

While adipose tissue macrophages, like classically activated macrophages, are inflamed they also have characteristics of alternatively activated macrophages. These M2-like characteristics include the expression of surface markers of alternative activation, remodeling of the extracellular matrix, promoting angiogenesis, and disposal of apoptotic adipocytes. During obesity, adipose tissue macrophages express cell surface markers associated with alternative activation (CD150, CD163, CD206) [28, 29]. They also produce collagen and the matrix remodeling proteins, matrix metalloproteases, associated with the reparative functions of M2 macrophages [28]. Like M2 macrophages, they respond to adipose tissue hypoxia by promoting the formation of new blood vessel networks [30, 31]. Finally, adipose tissue macrophages perform the vital function of clearing dead adipocytes and maintaining the tissue's ability to store more incoming fat [32–34]. Clearly, adipose tissue macrophages perform many beneficial functions in addition to their inflammatory pathogenic role during obesity.

Investigators have begun to understand macrophage phenotypes outside of the paradigm of M1 and M2. Adipose tissue macrophages are just one example of this trend that includes artery wall macrophages in atherosclerosis and macrophages exposed to high salt conditions, amongst others [35–37]. As mentioned above, obesity causes a phenotypic switch in macrophages that have characteristics of both classically and alternatively activated macrophages. This phenotype has been termed "metabolic activation" [37–39]. Metabolic activation (MMe) refers to the macrophage response to free FA prevalent in obese adipose tissue [37, 40–42]. The activation phenotype is proinflammatory and dependent on lysosome activity [43]. MMe macrophages promote detrimental insulin resistance while also beneficially clearing dead adipocytes. Overall, adipose tissue macrophages strive to maintain tissue homeostasis during obesity but also produce negative effects in the process.

In return, the metabolism of macrophages is affected by the status of the adipose tissue. In the M1/M2 paradigm it is well established that classically activated macrophages favor glycolytic energy sources whereas alternatively activated macrophages favor oxidative phosphorylation [44, 45]. Inhibiting glycolysis not only caused macrophages to favor oxidative phosphorylation, it reduces classical activation and inflammation [44]. Additionally, blocking FA oxidation pushes macrophages towards an inflammatory phenotype [46]. While lipid utilization and oxidative phosphorylation are associated with an anti-inflammatory phenotype, excessive lipid loading actually promotes inflammation. For example, during weight loss, when adipocyte lipolysis is elevated, adipose tissue macrophages become lipid loaded and metabolically inflamed [47]. Another major regulator of macrophage metabolism is hypoxia, prevalent in obese adipose tissue, which pushes macrophages towards

an inflammatory glycolytic phenotype [48, 49]. Overall, different metabolic pathways predispose macrophages to pro or anti-inflammatory phenotypes. While there is ample lipid available to promote oxidative phosphorylation and an anti-inflammatory phenotype, the confluence of excessive lipid loading and hypoxia pushes adipose tissue macrophages towards an inflammatory phenotype during obesity.

While macrophages are a major source of inflammation, they are not the only source. Macrophages operate in a network with other cells of the innate immune system as well as the adaptive immune system.

9.4 Other Innate Immune Cells

Innate lymphoid cells (ILCs) are a relatively recently discovered cell type. Evidence suggests that they play a role in promoting insulin sensitivity by maintaining anti-inflammatory populations of adipose tissue eosinophils and macrophages [50]. Interestingly, other works have shown that eosinophils migrate to the adipose tissue during obesity favoring the microenvironment to adipocyte maturation, Th2 cytokines (increased production of IL-4 and IL-13) and glucose tolerance [51]. On the other side of the inflammatory profile, the natural killer cells are known to infiltrate in the adipose tissue during obesity, increasing in number and producing higher concentrations of IFN-γ [52]. It is important to note that the development of insulin resistance is very complex and is far from being fully understood. Much more investigation is needed to understand the contribution of these different cell types.

9.5 B Cells

B cells are a major cell type in the adaptive immune system with the role of manufacturing and secreting antibodies. B cell populations have been identified in adipose depots and produce anti-inflammatory cytokines in the lean state [53]. Like macrophages, their numbers increase during obesity and they appear to promote inflammation and insulin resistance during obesity. Depleting B cells from adipose tissue improves insulin signaling and adoptive transfer of B cells into B cell null mice promotes insulin resistance [54, 55]. Interestingly, eliminating the major histocompatibility complex (MHC) in B cells also reduced insulin resistance [54]. This would suggest that there may be some obesity-associated antigen that B cells are presenting to T cells.

9.6 T Cells

The second major cell type of the adaptive immune system is the T cell, with the general role of eliminating infected cells. In the lean state, adipose tissue hosts a population of regulatory T cells (Tregs) that are distinct from splenic Tregs. This population of anti-inflammatory Tregs decreases during obesity [56]. However, if they are artificially maintained with exogenous cytokines the population can persist and is associated with improved insulin sensitivity [57, 58]. During obesity there is an increase in both CD4 + and CD8 + T cells in adipose tissue and both of these populations have been shown to contribute to insulin resistance [59, 60]. The driver of this T cell accumulation is still being investigated; though a combination of stress signals from adipocytes and macrophages is likely to be responsible. Infiltrating T cells promote inflammation and insulin resistance in adipose tissue.

It's well established that macrophages are very closely involved in the development of insulin resistance. However, investigations into the adaptive immune system demonstrate that it also contributes to the development of metabolic disease. From this we can conclude that adipose tissue is a complex microenvironment and that its dysfunction during obesity relies on many factors. In the final section of this chapter we will discuss the implications of inflammation in the development of metabolic syndrome in humans.

9.7 Adipose Tissue Inflammation

Obesity is associated with chronic inflammation in humans. In this section we will discuss the nature of this inflammation and its contribution to the development of two aspects of metabolic syndrome: insulin resistance and heart disease. We will also discuss the current findings of clinical trials for therapies targeting this inflammation to combat the ongoing epidemic of metabolic syndrome.

9.8 Inflammation and Type 2 Diabetes

T2D is a chronic disease that affects more than 500 million people worldwide and is predicted to continue to increase particularly in low-income countries [61]. T2D is characterized by a decreased response to the hormone insulin in the peripheral tissues. This decreased response requires the pancreas to overproduce insulin to maintain safe blood glucose levels [62]. The state of hyperinsulinemia with maintained normoglycemia is classified as prediabetes. As the disease progresses and insulin resistance worsens, patients develop both chronic hyperinsulinemia and hyperglycemia [62] that is characteristic of T2D. The chronic hyperglycemia of T2D increases patient risk for a wide variety of complications including, microvascular damage, sensory

neuropathy, amputation, heart attack, stroke, and death [63]. Moreover, it was found that every 1% improvement in glycemic control was associated with a 21% decrease in risk for diabetes related death [63].

Another hallmark of T2D is chronic systemic inflammation. This inflammation engages the same cytokines as infectious inflammation. However, the levels of obesity driven inflammation are much lower than the typical acute phase response to infection [64]. For example, levels of the inflammatory cytokine IL-6 levels in patients with sepsis are almost 20 times higher than patients with T2D [65, 66]. These low levels of chronic inflammation include soluble factors such as IL-6, (TNF-α), C reactive protein (CRP), and monocyte chemoattractant protein 1 (MCP-1) [64]. These elevated levels of inflammation correlate with insulin resistance in patients and have been shown to disrupt the pathway of insulin signaling on a cellular level [65, 67–70].

Investigators have used the power of mouse genetics to demonstrate a mechanistic link between inflammatory pathways and insulin resistance. Knocking out inflammatory signaling pathways in mice conveyed a protection against high-fat diet induced insulin resistance [26, 71, 72]. Additionally, blocking the inflammatory cytokines themselves protects mice from hyperglycemia and insulin resistance [73, 74]. TNF-α is the best understood mechanism by which an inflammatory cytokine directly causes insulin resistance. In typical insulin signaling the insulin receptor phosphorylates tyrosines on insulin receptor substrate-1 (IRS-1). Elevated levels of TNF-α phosphorylates serines on IRS-1 that prevent the phosphorylation of the crucial tyrosines. Thus, the proper signaling cascade is halted and the cell is unable to respond to extracellular insulin. Another inflammatory cytokine that has been linked to insulin resistance is IL-1β which is produced by the NLRP3 inflammasome in response to signals of cellular stress [75]. Knocking out the NLRP3 inflammasome complex prevents insulin resistance in obese mice. Moreover, IL-1β blockade improves insulin sensitivity in obese humans [76, 77]. Similar to TNF-α, investigators have shown that treating adipocytes with IL-1β causes the downregulation of IRS-1 [78]. In both cases, inflammatory cytokines interrupted IRS-1 signaling and, as a result, impair the adipocyte's response to insulin.

Overall, obesity promotes low levels of chronic inflammation that interferes with insulin signaling to cause insulin resistance. This insulin resistance can progress over time to T2D which puts patients at risk for organ damage and early death. Investigators are now targeting these inflammatory cytokines in an attempt to slow the development of insulin resistance and organ damage in diabetic patients.

9.9 Inflammation and Heart Disease

A second obesity-associated disease that makes up the metabolic syndrome is cardiovascular disease. As of 2017, cardiovascular disease was the leading cause of death for both men [1 in 4] and women (1 in 5) in the United States (79, 80). Many instances of cardiovascular disease are caused by the development of lipid plaques

within the wall of the blood vessel: termed atherosclerosis. Here we will deal with the contribution of obesity and inflammation to the development of atherosclerosis.

Atherosclerosis develops when lipids and lipoproteins invade the blood vessel wall and cause inflammation [81, 82]. These sites of inflammation are invaded by T cells and macrophages which can then proliferate locally in the plaque [83]. Over years of inflammation the smooth muscle of the blood vessel begins to invade the plaque which can become fibrotic [84]. Eventually, a necrotic core can develop [85]. As the plaque develops it can become unstable and susceptible to eruption which can cause life-threatening heart attacks and strokes if they occur in crucial arteries [85].

Inflammation is closely tied to the development of atherosclerosis as the initiating events involve inflammation of endothelial cells and the recruitment of inflammatory immune cells. In patients with unstable coronary artery disease high levels of C-reactive protein and interleukin 6 (IL-6) are strongly related to risk of death from cardiac issues [86, 87]. In mouse models of atherosclerosis, inflammatory cytokines promote many processes in plaque development including immune cell infiltration and fibrous cap development [88]. Preventing inflammatory immune infiltration or knocking out inflammatory cytokines protects mice from lesion development.

Overall, atherosclerosis is a complex inflammatory environment in which both systemic and local plaque inflammation interact with one another to progress the disease. Because of its prevalence and lethality, heart disease is still a major area of investigation (Figs. 1 and 2).

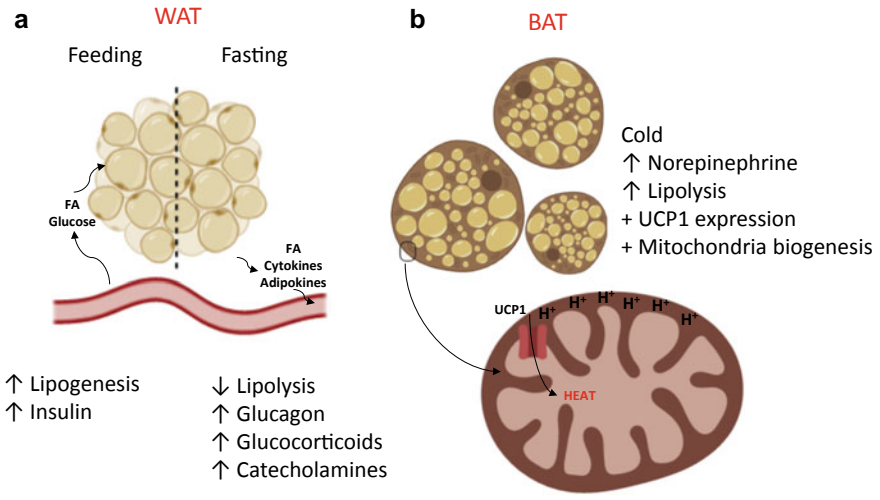


Fig. 1 **a** White adipose tissue is a central metabolic organ that acts as a reserve of energy while simultaneously acting as an endocrine organ that participates in regulating systemic energy homeostasis. **b** Brown adipose tissue responds to input from the central nervous system and uses stored lipid to produce heat.

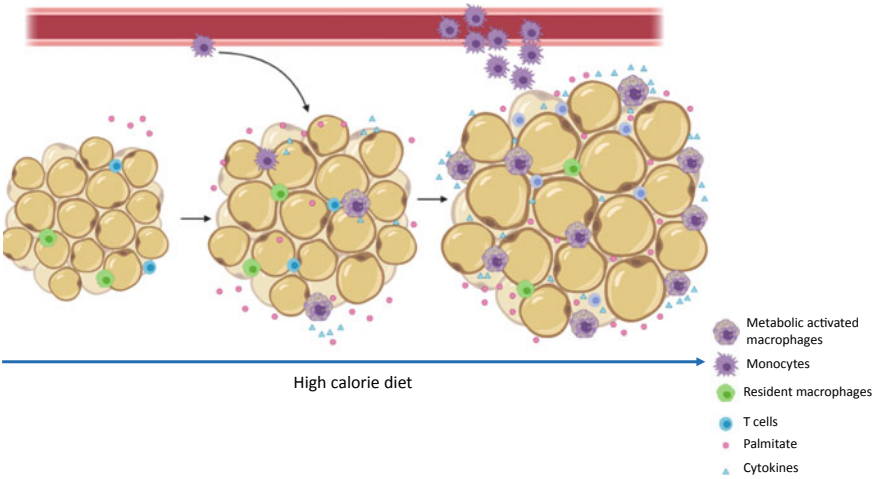


Fig. 2 During weight gain, adipocytes secrete free fatty acids, adipokines and cytokines, which activate and recruit immune cells. Macrophages migrate to the adipose tissue increasing the inflammatory profile and phagocyting the dead adipocytes

9.10 Human Trials in Anti-Inflammatories

Patients with metabolic syndrome have a predicted increased risk of all-cause mortality of 46% [89]. This substantial risk has made the development of a treatment for metabolic syndrome a high priority for investigators. For the past two decades investigators have pursued inflammation as a target in treating the metabolic syndrome because it is so tightly linked to multiple diseases within the larger syndrome. One of the earliest anti-inflammatory therapies is salicylate, also known as aspirin, which inhibits multiple inflammatory kinases. In clinical trials, patients treated with aspirin saw improvement in their blood glucose levels [90]. However, aspirin has well-known limitations including gastrointestinal irritation and blood thinning properties.

More recent drugs target specific proinflammatory cytokines or their receptors. In mouse studies blocking a handful of cytokine targets showed promising improvements in insulin sensitivity and atherosclerotic lesion development. Unfortunately, trials in humans were not consistent with those in mice. These human trials often ended with slight improvements in glucose but no change in insulin sensitivity.

For example, interleukin-1 (IL-1) antagonist treatment showed improvement in blood glucose but no difference in insulin resistance [91]. Additionally, administering an anti-interleukin 1 antibody improved incidences of non-fatal heart attack but did leave patients more susceptible fatal infection [92]. Blocking TNF-α similarly showed a reduced rate of cardiovascular disease events [93]. However, like IL-1 blockade, TNF-α blockers showed limited improvement in insulin sensitivity

in humans [94, 95]. Unlike interleukin 1, blocking IL-6 in autoimmune patients demonstrated improvements in fasting insulin [96].

The discovery of inflammation as a link between obesity and metabolic syndrome led to a new optimism about treatments. However, while mouse studies were positive for anti-inflammatory therapies, human trials were less promising. It is also important to consider their immunosuppressive effects on host defense and the danger that could pose with long term treatments. The failure of anti-inflammatory therapies to relieve metabolic syndrome does not negate the fact that the immune system is intimately involved in the development of metabolic syndrome. Obesity-associated inflammation consists of a spectrum of inflammatory cytokines and these studies confirm that blocking a single cytokine is not sufficient to reverse the metabolic syndrome. Rather, inhibiting the initiating events of inflammatory pathways to reduce the entire spectrum of inflammatory cytokines is a new promising area of research against the metabolic syndrome.

References

1. Ottaviani E, Malagoli D, Franceschi C (2011) The evolution of the adipose tissue: a neglected enigma. Gen Comp Endocrinol 174(1):1–4
2. Nye C, Kim J, Kalhan SC, Hanson RW (2008) Reassessing triglyceride synthesis in adipose tissue. Trends Endocrinol Metab 19(10):356–361
3. Dimitriadis G, Mitrou P, Lambadiari V, Maratou E, Raptis SA (2011) Insulin effects in muscle and adipose tissue. Diabetes Res Clin Pract 93(Suppl 1):S52–S59
4. Ahmadian M, Wang Y, Sul HS (2010) Lipolysis in adipocytes. Int J Biochem Cell Biol 42(5):555–559
5. Luo L, Liu M (2016) Adipose tissue in control of metabolism. J Endocrinol 231(3):R77-r99
6. Rosen ED, Spiegelman BM (2014) What We Talk About When We Talk About Fat*. Cell 156:20–44
7. Cannon B, Nedergaard J (2004) Brown adipose tissue: function and physiological significance. Physiol Rev 84(1):277–359
8. Choe SS, Huh JY, Hwang IJ, Kim JI, Kim JB. Adipose Tissue Remodeling: Its Role in Energy Metabolism and Metabolic Disorders. Front Endocrinol (Lausanne). 2016;7.
9. Shinoda K, Luijten IH, Hasegawa Y, Hong H, Sonne SB, Kim M et al (2015) Genetic and functional characterization of clonally derived adult human brown adipocytes. Nat Med 21(4):389–394
10. Cohen P, Spiegelman BM (2015) Brown and Beige Fat: Molecular Parts of a Thermogenic Machine. Diabetes 64(7):2346–2351
11. Seale P, Bjork B, Yang W, Kajimura S, Chin S, Kuang S et al (2008) PRDM16 controls a brown fat/skeletal muscle switch. Nature 454(7207):961–967
12. Min SY, Kady J, Nam M, Rojas-Rodriguez R, Berkenwald A, Kim JH et al (2016) Human "brite/beige" adipocytes develop from capillary networks, and their implantation improves metabolic homeostasis in mice. Nat Med 22(3):312–318
13. Fasshauer M, Bluher M (2015) Adipokines in health and disease. Trends Pharmacol Sci 36(7):461–470
14. Bjørbæk C (2009) Central Leptin Receptor Action and Resistance in Obesity. J Investig Med 57(7):789–794
15. Monteiro L, Pereira J, Palhinha L, Moraes-Vieira PMM. Leptin in the regulation of the immunometabolism of adipose tissue-macrophages. J Leukoc Biol. 2019.

16. Munzberg H, Flier JS, Bjorbaek C (2004) Region-specific leptin resistance within the hypothalamus of diet-induced obese mice. Endocrinology 145(11):4880–4889
17. Kubota N, Yano W, Kubota T, Yamauchi T, Itoh S, Kumagai H et al (2007) Adiponectin stimulates AMP-activated protein kinase in the hypothalamus and increases food intake. Cell Metab 6(1):55–68
18. Yamauchi T, Kamon J, Waki H, Terauchi Y, Kubota N, Hara K et al (2001) The fat-derived hormone adiponectin reverses insulin resistance associated with both lipoatrophy and obesity. Nat Med 7(8):941–946
19. Nawrocki AR, Rajala MW, Tomas E, Pajvani UB, Saha AK, Trumbauer ME et al (2006) Mice lacking adiponectin show decreased hepatic insulin sensitivity and reduced responsiveness to peroxisome proliferator-activated receptor gamma agonists. J Biol Chem 281(5):2654–2660
20. Gordon S (2016) Phagocytosis: The Legacy of Metchnikoff. Cell 166(5):1065–1068
21. Epelman S, Lavine KJ, Randolph GJ (2014) Origin and Functions of Tissue Macrophages. Immunity 41(1):21–35
22. Gerhardt T, Ley K. Monocyte trafficking across the vessel wall. Cardiovasc Res. 1072015. p. 321–30.
23. Boutens L, Stienstra R. Adipose tissue macrophages: going off track during obesity. Diabetologia. 592016. p. 879–94.
24. Weisberg SP, McCann D, Desai M, Rosenbaum M, Leibel RL, Ferrante AW Jr (2003) Obesity is associated with macrophage accumulation in adipose tissue. J Clin Invest 112(12):1796–1808
25. Patsouris D, Li PP, Thapar D, Chapman J, Olefsky JM, Neels JG (2008) Ablation of CD11c-positive cells normalizes insulin sensitivity in obese insulin resistant animals. Cell Metab 8(4):301–309
26. Han MS, Jung DY, Morel C, Lakhani SA, Kim JK, Flavell RA et al (2013) JNK expression by macrophages promotes obesity-induced insulin resistance and inflammation. Science 339(6116):218–222
27. Saberi M, Woods NB, de Luca C, Schenk S, Lu JC, Bandyopadhyay G et al (2009) Hematopoietic cell-specific deletion of toll-like receptor 4 ameliorates hepatic and adipose tissue insulin resistance in high-fat-fed mice. Cell Metab 10(5):419–429
28. Zeyda M, Farmer D, Todoric J, Aszmann O, Speiser M, Gyori G et al (2007) Human adipose tissue macrophages are of an anti-inflammatory phenotype but capable of excessive pro-inflammatory mediator production. Int J Obes (Lond) 31(9):1420–1428
29. Spencer M, Yao-Borengasser A, Unal R, Rasouli N, Gurley CM, Zhu B et al (2010) Adipose tissue macrophages in insulin-resistant subjects are associated with collagen VI and fibrosis and demonstrate alternative activation. Am J Physiol Endocrinol Metab 299(6):E1016–E1027
30. Pang C, Gao Z, Yin J, Zhang J, Jia W, Ye J (2008) Macrophage infiltration into adipose tissue may promote angiogenesis for adipose tissue remodeling in obesity. Am J Physiol Endocrinol Metab 295(2):E313–E322
31. Cho CH, Koh YJ, Han J, Sung HK, Jong Lee H, Morisada T et al (2007) Angiogenic role of LYVE-1-positive macrophages in adipose tissue. Circ Res 100(4):e47-57
32. Haka AS, Barbosa-Lorenzi VC, Lee HJ, Falcone DJ, Hudis CA, Dannenberg AJ et al (2016) Exocytosis of macrophage lysosomes leads to digestion of apoptotic adipocytes and foam cell formation. J Lipid Res 57(6):980–992
33. Coats BR, Schoenfelt KQ, Barbosa-Lorenzi VC, Peris E, Cui C, Hoffman A et al (2017) Metabolically Activated Adipose Tissue Macrophages Perform Detrimental and Beneficial Functions during Diet-Induced Obesity. Cell Rep 20(13):3149–3161
34. Nishimura S, Manabe I, Nagasaki M, Hosoya Y, Yamashita H, Fujita H et al (2007) Adipogenesis in obesity requires close interplay between differentiating adipocytes, stromal cells, and blood vessels. Diabetes 56(6):1517–1526
35. Reardon CA, Lingaraju A, Schoenfelt KQ, Zhou G, Cui C, Jacobs-El H et al (2018) Obesity and Insulin Resistance Promote Atherosclerosis through an IFNgamma-Regulated Macrophage Protein Network. Cell Rep 23(10):3021–3030
36. Zhang WC, Zheng XJ, Du LJ, Sun JY, Shen ZX, Shi C et al (2015) High salt primes a specific activation state of macrophages, M(Na). Cell Res 25(8):893–910

37. Kratz M, Coats BR, Hisert KB, Hagman D, Mutskov V, Peris E et al (2014) Metabolic dysfunction drives a mechanistically distinct proinflammatory phenotype in adipose tissue macrophages. Cell Metab 20(4):614–625
38. Boutens L, Hooiveld GJ, Dhingra S, Cramer RA, Netea MG, Stienstra R (2018) Unique metabolic activation of adipose tissue macrophages in obesity promotes inflammatory responses. Diabetologia 61(4):942–953
39. Kane H, Lynch L. Innate Immune Control of Adipose Tissue Homeostasis. Trends Immunol. 2019.
40. Suganami T, Nishida J, Ogawa Y (2005) A paracrine loop between adipocytes and macrophages aggravates inflammatory changes: role of free fatty acids and tumor necrosis factor alpha. Arterioscler Thromb Vasc Biol 25(10):2062–2068
41. Nguyen MT, Favelyukis S, Nguyen AK, Reichart D, Scott PA, Jenn A et al (2007) A subpopulation of macrophages infiltrates hypertrophic adipose tissue and is activated by free fatty acids via Toll-like receptors 2 and 4 and JNK-dependent pathways. J Biol Chem 282(48):35279–35292
42. Snodgrass RG, Huang S, Choi IW, Rutledge JC, Hwang DH (2013) Inflammasome-mediated secretion of IL-1βeta in human monocytes through TLR2 activation; modulation by dietary fatty acids. J Immunol 191(8):4337–4347
43. Xu X, Grijalva A, Skowronski A, van Eijk M, Serlie MJ, Ferrante AW Jr (2013) Obesity activates a program of lysosomal-dependent lipid metabolism in adipose tissue macrophages independently of classic activation. Cell Metab 18(6):816–830
44. Freemerman AJ, Johnson AR, Sacks GN, Milner JJ, Kirk EL, Troester MA et al (2014) Metabolic reprogramming of macrophages: glucose transporter 1 (GLUT1)-mediated glucose metabolism drives a proinflammatory phenotype. J Biol Chem 289(11):7884–7896
45. Verdeguer F, Aouadi M (2017) Macrophage heterogeneity and energy metabolism. Exp Cell Res 360(1):35–40
46. Johnson AR, Qin Y, Cozzo AJ, Freemerman AJ, Huang MJ, Zhao L et al (2016) Metabolic reprogramming through fatty acid transport protein 1 (FATP1) regulates macrophage inflammatory potential and adipose inflammation. Mol Metab. 5(7):506–526
47. Kosteli A, Sugaru E, Haemmerle G, Martin JF, Lei J, Zechner R et al (2010) Weight loss and lipolysis promote a dynamic immune response in murine adipose tissue. J Clin Invest 120(10):3466–3479
48. Snodgrass RG, Boss M, Zezina E, Weigert A, Dehne N, Fleming I et al (2016) Hypoxia Potentiates Palmitate-induced Pro-inflammatory Activation of Primary Human Macrophages. J Biol Chem 291(1):413–424
49. Ryan S (2017) Adipose tissue inflammation by intermittent hypoxia: mechanistic link between obstructive sleep apnoea and metabolic dysfunction. J Physiol 595(8):2423–2430
50. Molofsky AB, Nussbaum JC, Liang HE, Van Dyken SJ, Cheng LE, Mohapatra A et al (2013) Innate lymphoid type 2 cells sustain visceral adipose tissue eosinophils and alternatively activated macrophages. J Exp Med 210(3):535–549
51. Lee EH, Itan M, Jang J, Gu HJ, Rozenberg P, Mingler MK et al (2018) Eosinophils support adipocyte maturation and promote glucose tolerance in obesity. Sci Rep 8(1):9894
52. O'Rourke RW, Metcalf MD, White AE, Madala A, Winters BR, Maizlin II et al (2009) Depot-specific differences in inflammatory mediators and a role for NK cells and IFN-gamma in inflammation in human adipose tissue. Int J Obes (Lond) 33(9):978–990
53. Nishimura S, Manabe I, Takaki S, Nagasaki M, Otsu M, Yamashita H et al (2013) Adipose Natural Regulatory B Cells Negatively Control Adipose Tissue Inflammation. Cell Metab 18(5):759–766
54. Winer DA, Winer S, Shen L, Wadia PP, Yantha J, Paltser G et al (2011) B cells promote insulin resistance through modulation of T cells and production of pathogenic IgG antibodies. Nat Med 17(5):610–617
55. Ying W, Wollam J, Ofrecio JM, Bandyopadhyay G, El Ouarrat D, Lee YS et al (2017) Adipose tissue B2 cells promote insulin resistance through leukotriene LTB4/LTB4R1 signaling. J Clin Invest 127(3):1019–1030

56. Feuerer M, Herrero L, Cipolletta D, Naaz A, Wong J, Nayer A et al (2009) Lean, but not obese, fat is enriched for a unique population of regulatory T cells that affect metabolic parameters. Nat Med 15(8):930–939
57. Vasanthakumar A, Moro K, Xin A, Liao Y, Gloury R, Kawamoto S et al (2015) The transcriptional regulators IRF4, BATF and IL-33 orchestrate development and maintenance of adipose tissue-resident regulatory T cells. Nat Immunol 16(3):276–285
58. Han JM, Wu D, Denroche HC, Yao Y, Verchere CB, Levings MK (2015) IL-33 Reverses an Obesity-Induced Deficit in Visceral Adipose Tissue ST2+ T Regulatory Cells and Ameliorates Adipose Tissue Inflammation and Insulin Resistance. J Immunol 194(10):4777–4783
59. Nishimura S, Manabe I, Nagasaki M, Eto K, Yamashita H, Ohsugi M et al (2009) CD8+ effector T cells contribute to macrophage recruitment and adipose tissue inflammation in obesity. Nat Med 15(8):914–920
60. Kintscher U, Hartge M, Hess K, Foryst-Ludwig A, Clemenz M, Wabitsch M et al (2008) T-lymphocyte infiltration in visceral adipose tissue: a primary event in adipose tissue inflammation and the development of obesity-mediated insulin resistance. Arterioscler Thromb Vasc Biol 28(7):1304–1310
61. KAISER AB, ZHANG N, PLUIJM WVD. Global Prevalence of Type 2 Diabetes over the Next Ten Years (2018–2028). 2018.
62. Kahn BB, Flier JS. Obesity and insulin resistance. J Clin Invest. 1062000. p. 473–81.
63. Stratton IM, Adler AI, Neil HAW, Matthews DR, Manley SE, Cull CA, et al. Association of glycaemia with macrovascular and microvascular complications of type 2 diabetes (UKPDS 35): prospective observational study. 2000.
64. Castoldi A, Naffah de Souza C, Camara NO, Moraes-Vieira PM. The Macrophage Switch in Obesity Development. Front Immunol. 2015;6:637.
65. Fontana L, Eagon JC, Trujillo ME, Scherer PE, Klein S (2007) Visceral fat adipokine secretion is associated with systemic inflammation in obese humans. Diabetes 56(4):1010–1013
66. Bernard GR, Vincent JL, Laterre PF, LaRosa SP, Dhainaut JF, Lopez-Rodriguez A et al (2001) Efficacy and safety of recombinant human activated protein C for severe sepsis. N Engl J Med 344(10):699–709
67. Wellen KE, Hotamisligil GS (2005) Inflammation, stress, and diabetes. J Clin Invest 115(5):1111–1119
68. Hotamisligil GS, Arner P, Caro JF, Atkinson RL, Spiegelman BM (1995) Increased adipose tissue expression of tumor necrosis factor-alpha in human obesity and insulin resistance. J Clin Invest 95(5):2409–2415
69. Hotamisligil GS, Spiegelman BM (1994) Tumor necrosis factor alpha: a key component of the obesity-diabetes link. Diabetes 43(11):1271–1278
70. Kern PA, Ranganathan S, Li C, Wood L, Ranganathan G (2001) Adipose tissue tumor necrosis factor and interleukin-6 expression in human obesity and insulin resistance. Am J Physiol Endocrinol Metab 280(5):E745–E751
71. Shi H, Kokoeva MV, Inouye K, Tzameli I, Yin H, Flier JS (2006) TLR4 links innate immunity and fatty acid-induced insulin resistance. J Clin Invest 116(11):3015–3025
72. Himes RW, Smith CW (2010) Tlr2 is critical for diet-induced metabolic syndrome in a murine model. Faseb j 24(3):731–739
73. Uysal KT, Wiesbrock SM, Marino MW, Hotamisligil GS (1997) Protection from obesity-induced insulin resistance in mice lacking TNF-alpha function. Nature 389(6651):610–614
74. O'Rourke RW, White AE, Metcalf MD, Winters BR, Diggs BS, Zhu X et al (2012) Systemic inflammation and insulin sensitivity in obese IFN-gamma knockout mice. Metabolism 61(8):1152–1161
75. Kuida K, Lippke JA, Ku G, Harding MW, Livingston DJ, Su MS et al (1995) Altered cytokine export and apoptosis in mice deficient in interleukin-1 beta converting enzyme. Science 267(5206):2000–2003
76. Stienstra R, van Diepen JA, Tack CJ, Zaki MH, van de Veerdonk FL, Perera D et al (2011) Inflammasome is a central player in the induction of obesity and insulin resistance. Proc Natl Acad Sci U S A 108(37):15324–15329

77. Vandanmagsar B, Youm YH, Ravussin A, Galgani JE, Stadler K, Mynatt RL et al (2011) The NLRP3 inflammasome instigates obesity-induced inflammation and insulin resistance. Nat Med 17(2):179–188
78. Jager J, Gremeaux T, Cormont M, Le Marchand-Brustel Y, Tanti JF (2007) Interleukin-1beta-induced insulin resistance in adipocytes through down-regulation of insulin receptor substrate-1 expression. Endocrinology 148(1):241–251
79. Women and Heart Disease Prevention - Women's Health - CDC. 2019.
80. @CDCgov. Men and Heart Disease | cdc.gov. 2019.
81. Skalen K, Gustafsson M, Rydberg EK, Hulten LM, Wiklund O, Innerarity TL et al (2002) Subendothelial retention of atherogenic lipoproteins in early atherosclerosis. Nature 417(6890):750–754
82. Leitinger N (2003) Oxidized phospholipids as modulators of inflammation in atherosclerosis. Curr Opin Lipidol 14(5):421–430
83. Robbins CS, Hilgendorf I, Weber GF, Theurl I, Iwamoto Y, Figueiredo JL et al (2013) Local proliferation dominates lesional macrophage accumulation in atherosclerosis. Nat Med 19(9):1166–1172
84. Newby AC, Zaltsman AB (1999) Fibrous cap formation or destruction–the critical importance of vascular smooth muscle cell proliferation, migration and matrix formation. Cardiovasc Res 41(2):345–360
85. Gonzalez L, Trigatti BL (2017) Macrophage Apoptosis and Necrotic Core Development in Atherosclerosis: A Rapidly Advancing Field with Clinical Relevance to Imaging and Therapy. Can J Cardiol 33(3):303–312
86. Lindahl B, Toss H, Siegbahn A, Venge P, Wallentin L. Markers of myocardial damage and inflammation in relation to long-term mortality in unstable coronary artery disease. FRISC Study Group. Fragmin during Instability in Coronary Artery Disease. N Engl J Med. 2000;343(16):1139–47.
87. Biasucci LM, Vitelli A, Liuzzo G, Altamura S, Caligiuri G, Monaco C et al (1996) Elevated levels of interleukin-6 in unstable angina. Circulation 94(5):874–877
88. Nageh MF, Sandberg ET, Marotti KR, Lin AH, Melchior EP, Bullard DC et al (1997) Deficiency of inflammatory cell adhesion molecules protects against atherosclerosis in mice. Arterioscler Thromb Vasc Biol 17(8):1517–1520
89. Wu SH, Liu Z, Ho SC (2010) Metabolic syndrome and all-cause mortality: a meta-analysis of prospective cohort studies. Eur J Epidemiol 25(6):375–384
90. Goldfine AB, Fonseca V, Jablonski KA, Chen YD, Tipton L, Staten MA et al (2013) Salicylate (salsalate) in patients with type 2 diabetes: a randomized trial. Ann Intern Med 159(1):1–12
91. Larsen CM, Faulenbach M, Vaag A, Volund A, Ehses JA, Seifert B et al (2007) Interleukin-1-receptor antagonist in type 2 diabetes mellitus. N Engl J Med 356(15):1517–1526
92. Ridker PM, Everett BM, Thuren T, MacFadyen JG, Chang WH, Ballantyne C et al (2017) Antiinflammatory Therapy with Canakinumab for Atherosclerotic Disease. N Engl J Med 377(12):1119–1131
93. Jacobsson LT, Turesson C, Gulfe A, Kapetanovic MC, Petersson IF, Saxne T et al (2005) Treatment with tumor necrosis factor blockers is associated with a lower incidence of first cardiovascular events in patients with rheumatoid arthritis. J Rheumatol 32(7):1213–1218
94. Martinez-Abundis E, Reynoso-von Drateln C, Hernandez-Salazar E, Gonzalez-Ortiz M (2007) Effect of etanercept on insulin secretion and insulin sensitivity in a randomized trial with psoriatic patients at risk for developing type 2 diabetes mellitus. Arch Dermatol Res 299(9):461–465
95. Dominguez H, Storgaard H, Rask-Madsen C, Steffen Hermann T, Ihlemann N, Baunbjerg Nielsen D et al (2005) Metabolic and vascular effects of tumor necrosis factor-alpha blockade with etanercept in obese patients with type 2 diabetes. J Vasc Res 42(6):517–525
96. Schultz O, Oberhauser F, Saech J, Rubbert-Roth A, Hahn M, Krone W, et al. Effects of inhibition of interleukin-6 signalling on insulin sensitivity and lipoprotein (a) levels in human subjects with rheumatoid diseases. PLoS One. 2010;5(12):e14328.

Immune Regulation of Adipose Tissue Browning

Andréa L. Rocha, Luiz Osório Leiria, Tim J. Schulz, and Marcelo A. Mori

1 Introduction

Adipose tissue is a central player in the pathophysiology of a number of metabolic diseases, including obesity, type 2 diabetes, non-alcoholic fatty liver disease (NAFLD) and atherosclerosis [1, 2]. The classical view of the role of adipose tissue is that it serves as a reservoir for storing energy as triglycerides, thus releasing free fatty acids by demand under negative energy balance conditions [3, 4]. This apparently simple landscape started to gain complexity in the 90's, when adipose tissue was found to secrete leptin and several other cytokines linked to insulin resistance and obesity, including tumor necrosis factor alpha (TNFα) [5–9]. These groundbreaking discoveries implied that adipose tissue could at the same time serve as an endocrine organ and a source of pro-inflammatory molecules driving the development of insulin resistance.

The importance of the immune system for the maintenance of adipocyte functions is reinforced by the presence of a wide variety of immune cells within this tissue [10–13]. Indeed, several studies have reinforced the strong and causal link between adipose tissue low-grade inflammation and insulin resistance [14, 15]. As

A. L. Rocha · L. O. Leiria · M. A. Mori (✉)
Department of Biochemistry and Tissue Biology, Institute of Biology, University of Campinas, Campinas, SP, Brazil
e-mail: morima@unicamp.br

T. J. Schulz
German Institute of Human Nutrition Potsdam-Rehbruecke (DIfE), Potsdam, Germany

German Center for Diabetes Research (DZD), München-Neuherberg, Germany

Institute of Nutritional Science, University of Potsdam, Potsdam-Rehbrücke, Nuthetal, Germany

L. O. Leiria
Department of Pharmacology, Ribeirão Preto Medical School, University of São Paulo, Ribeirão Preto-SP, Brazil

N. O. S. Camara et al. (eds.), *Essential Aspects of Immunometabolism in Health and Disease*, https://doi.org/10.1007/978-3-030-86684-6_11

will be discussed in this chapter, the interactions between the immune system and adipocytes are not restricted to the immunological disbalance underlying pathological conditions, but also helps maintaining adipose tissue homeostasis during normal physiology [15, 16], and underlies the phenotypic conversion of white to the beige fat under negative energy balance conditions, such as cold stress, caloric restriction and physical exercise [17–19].

Adipose tissue is distributed throughout the mammalian body in different fat depots, which display distinct and even opposite roles in energy metabolism, depending on the metabolic demand and the adipocyte type. Opposing the energy-storing role of the classical unilocular white adipose tissue (WAT), there are, to date, two documented thermogenic competent types of adipocytes in mammals: (i) the constitutive classical brown adipocytes, which comprise the brown adipose tissue (BAT); (ii) and the inducible beige adipocytes (or brite, from "brown in white"), that develop as clusters within white fat depots upon appropriate stimulation. Both beige and brown fat have the ability to burn substrates in response to cold stress in order to produce heat and defend body temperature, as a part of adaptive thermogenesis, which ultimately leads to enhanced energy expenditure. In order to be able to generate heat, these thermogenic adipocytes take up large amounts of fatty acids and glucose from the circulation, which are then immediately utilized for (i) the activation of the uncoupling protein 1 (UCP1); (ii) to sustain anaplerotic reactions needed to replenish ATP levels; (iii) to undergo oxidation; and (iii) to be incorporated in triglycerides to temporarily accumulate more fuel for further utilization [20]. These processes are stimulated mainly by β-adrenergic signaling [21]. In addition to its ability to increase energy expenditure, thermogenic fat also exerts endocrine functions by regulating lipid/glucose metabolism in other tissues through the secretion of bioactive factors, including proteins, lipids and exosomal miRNAs [22–24]. Due to these unique features, the induction of thermogenic fat is a compelling therapeutic target to fight diabetes or other metabolic diseases.

Although the browning process was first documented over 35 years ago [25], the molecular regulators and developmental origins of this process started to be deeply dissected only through the last decade [26–28]. During this period, studies have also demonstrated that adult humans have a pool of functional brown adipocytes [29–31]. Although there are many lines of evidence supporting the idea that the immune system participates in beige adipocyte formation, the exact mechanisms as well as the main players are still largely unknown. The first link between immune cells and browning was described in a study by Nguyen et al. [32], which demonstrated that alternative activation of macrophages was a key and limiting phenomenon leading to neuronal-independent browning of WAT. This study suggested that a population of alternatively activated M2 macrophages possess the capacity to locally biosynthesize and release catecholamines to drive beige fat induction. However, some years later another study showed that macrophages are actually unable to synthesize catecholamines, and that the browning process does not requires alternative activation of macrophages [33]. More recently, a study showed that sympathetic neuron-associated macrophages (SAMs) are able to take up norepinephrine through a solute carrier family 6 member 2 (SLC6A2) and metabolize it, thus counteracting sympathetic tone

and favoring weight gain [34]. Ablation of the *Slc6a2* in SAMs caused browning of white adipose tissue and increased thermogenesis, demonstrating that this population of macrophages actually counteracts the browning process.

Although the role of immune cells in adipose tissue remains a matter of debate, M2 macrophages and other immune cells such as type 2 innate lymphoid cells (ILC2) [35, 36] and γδT cells have been consistently shown to contribute to beige fat biogenesis by triggering distinct pathways [37]. Moreover, a number of cytokines has also been implicated in thermogenesis and in the browning process. In the next sections we will discuss in detail the main evidence, to date, linking immune cells and their secreted factors with adipose tissue browning.

2 Immune Cells in Adipose Tissue

Adipose tissue consists of adipocytes and a stromal-vascular fraction (SVF) containing a heterogeneous network of cells, including endothelial cells, stem cells, preadipocytes, fibroblasts, and a wide range of immune cells [38, 39]. These leukocytes are essentially present in all fat depots, spread throughout the tissue, in between adipocytes, along blood vessels, and as part of defined lymphoid structures [40]. In this part of the chapter we will describe the immune cells found in different adipose tissue depots and how they play a role in adipose tissue function (Fig. 1).

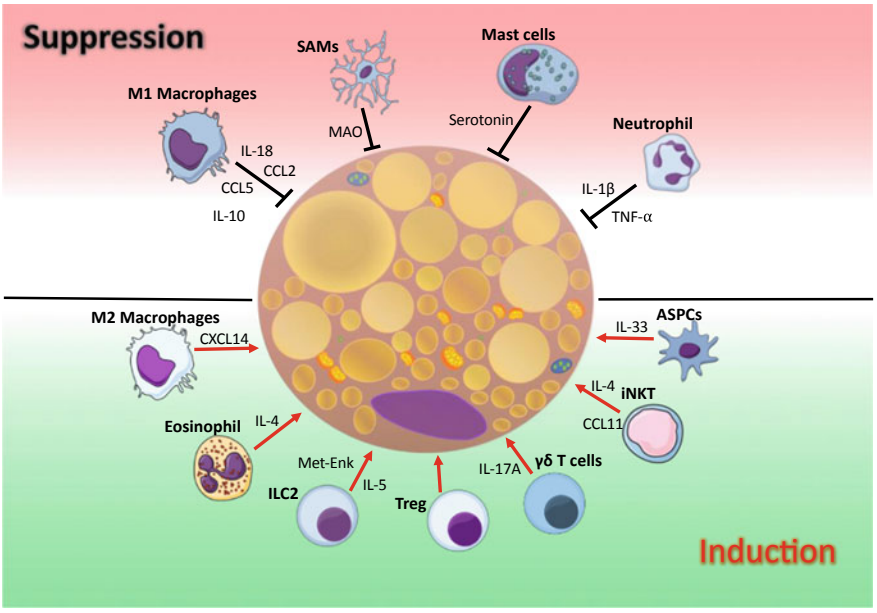


Fig. 1 Immune cells and mediators that control browning

2.1 Myeloid Cells

Macrophages are the most abundant immune cell type in adipose tissue. **Classically activated, pro-inflammatory M1 macrophages** are widely distributed across different WAT depots, with particular enrichment in visceral adipose tissue (VAT) during obesity [10]. Consistently, VAT accumulation strongly associates with the development of the metabolic syndrome [41–43], in part due to the pro-inflammatory nature of this depot, which is rich in **TNFα** and **IL-1β** [44–46]. This is further supported by the observation that aging results in increased accumulation of pro-inflammatory macrophages in VAT, in agreement with the notion that metabolic disease progression is exacerbated by age-related inflammatory processes, termed 'InflammAging', in adipose tissue [47]. On the other hand, **alternatively activated, anti-inflammatory M2 macrophages** are particularly abundant in the browning-prone subcutaneous fat depot. These macrophages are mainly stimulated by type II cytokines **IL-4** and **IL-13**, as well as by **IL-6**, which induces **IL-4Rα** [48, 49]. **Eosinophils** are thought to be the main IL-4-expressing cells in mouse WAT, and in their absence, M2 macrophages are greatly reduced [49]. Eosinophils are recruited in response to **IL-5**-mediated **type 2 innate lymphoid cell (ILC2)** activation (see below), which also contributes to differentiation of beige adipocytes [36, 50, 51]. Adipose tissue of lean mice contains a large population of resident eosinophils that plays an important role in glucose homeostasis and the regulation of adiposity and systemic insulin resistance [35, 51].

Macrophages are also present in BAT but at reduced numbers if compared to WAT [52, 53]. Analysis of the association between experimental obesity and inflammatory pathways in BAT has demonstrated that the transcript levels of several pro-inflammatory cytokines are increased during obesity in this tissue. The upregulated markers include **TNFα** and the chemokines **CCL2** (**MCP-1**) and **CCL5**, thus suggesting the recruitment of additional pro-inflammatory cells [54, 55]. Studies have indicated that cytokines such as **TNFα** and **IL-1β** inhibit β-adrenergic-induced *Ucp1* expression in brown adipocytes [56, 57], thus indicating that a pro-inflammatory milieu may limit the induction of thermogenesis in BAT. On the other hand, as mentioned above, anti-inflammatory pathways responsible for alternative activation of macrophages have also been reported to elicit a thermogenic response**.** These include **IL-4** signaling [58, 59]. Brown adipocytes also secrete factors that contribute to maintenance of thermogenesis via activation of macrophages. For example, the chemokine **CXCL14** is secreted by brown fat to sustain thermogenesis in response to cold stimulation via alternative activation of macrophages in brown and white adipose tissues [60]. Another study demonstrating the role of macrophages in BAT implicates these cells in the homeostatic maintenance of sympathetic innervation [61].

Neutrophils have been described in WAT, where they are present at a lower proportion (<1% of all immune cells), but increase with obesity, when they contribute to insulin resistance [62]. **Mast cells** are also increased in WAT of obese compared to lean individuals, and this is associated with impaired glucose intolerance. Conversely,

pharmacological stabilization or genetic ablation of mast cells ameliorate many effects of diet induced obesity and lead to increased energy expenditure, which has been linked to increased beige adipogenesis and expression of UCP1 in WAT [63]. This is further supported by a more recent study that demonstrated increased browning upon mast cell inactivation and consequent inhibition of **serotonin** release [64]. In contrast, recent studies have shown that mast cells in human subcutaneous adipose tissue respond to cold, release factors such as **histamine** and thus promote UCP1 expression in white adipocytes [65].

2.2 Lymphoid Cells

In addition to myeloid cells, adipose tissue has a wide variety of lymphocytes, including **T cells**, **B cells**, and **invariant natural killer (iNKT) cells.** When arriving in the adipose tissue from circulation, particularly during obesity, **CD8 + T cells** can be activated by inflammatory B cells to induce **IFN-γ** and **granzyme B** production, which in turn promote macrophage accumulation and insulin resistance [66, 67]. **CD4 + T regulatory (Treg) cells** are relatively abundant in VAT, but they reduce in number in mouse models of obesity [68]. Treg cells in VAT are maintained by **IL-33** signaling through **ST2** stimulation and **PPARγ** activation [69]. IL-33 administration leads to Treg expansion, which associates with metabolic improvements in obese mice [69], including induced thermogenesis [36, 70]. Consistent with the protective roles of Treg cells, depletion of Tregs in lean mice impairs insulin sensitivity and increases fasting blood glucose levels [71, 72]. On the other hand, aging leads to an over tenfold increase in Tregs in mouse VAT, while depletion of adipose tissue Tregs prevents age-associated metabolic dysfunction [11]. These results indicate that Tregs have different roles in obesity and aging.

T cells are also found in BAT. Medrikova et al. identified a particular sub-set of **Tregs** in BAT [73] which appear to be important for BAT function, given that systemic ablation of Tregs abrogates cold-induced thermogenesis and results in accumulation of pro-inflammatory macrophages in BAT. These findings were corroborated by Kälin et al., 2017 [74], who also shown that upon cold exposure or β3-adrenergic stimulation, CD4 + T cells express higher levels of Treg-related regulatory networks in a UCP1-dependent manner.

γδ T cells are found in visceral, subcutaneous and brown adipose tissues, where they produce **IL-17A**, which in turn regulates IL-33 production by adipose stromal cells [37]. Mice lacking γδ T cells or IL-17A have less IL-33 and less Treg cells in VAT. Given the critical role of IL-33 in thermogenesis [36, 70], these mice lack the ability to regulate core body temperature at cold exposure [37].

Like T cells and macrophages, **B cells** have also been shown to infiltrate VAT and manifest phenotypic changes during diet-induced obesity [75]. B cells control systemic and local adipose tissue inflammation during obesity-related insulin resistance via antigen presentation, cytokine secretion, and antibody production [67, 75]. B cells represent up to 30% of all immune cells in mouse BAT and increase in

this tissue with obesity [76]. B cell phenotypic plasticity is influenced by both norepinephrine [77] and adenosine [78], which have been shown to activate thermogenesis in BAT. However, no clear evidence for the role of B cells in adaptive thermogenesis has been demonstrated so far.

Invariant natural killer (iNKT) cells are also present in WAT where they promote an anti-inflammatory phenotype [79]. In mice, activation of adipose iNKT cells with alpha- galactosylceramide administration promotes weight loss associated with increased energy expenditure and browning of white fat [80]. Part of this effect is mediated by increased **FGF21**, which upregulates **CCL11** and leads to recruitment of eosinophils to subcutaneous adipose tissue where browning takes place through the mechanisms discussed above [80].

Both human and murine WAT contain considerable levels of **type-2 innate lymphoid cells (ILC2s)**. Moreover, ILC2 content in the visceral fat inversely correlates with body mass index in both humans and rodents [36]. ILC2s constitutively express the cytokine **IL-33 receptor ST2** and are strongly responsive to **IL-33**, but also respond to a lesser extent to other cytokines such as **IL-2**, **IL-7** and **IL-25** [36, 81]. Adipose stem and progenitor cells (ASPCs) were recently found to be the main source of IL-33 in WAT, while mesothelial cells act as an additional source of IL-33 specifically in VAT [82].

3 Immune Cell Mediators and Their Role in Browning

Studies have extensively highlighted the importance of anti-inflammatory, **type II cytokines** in regulating adaptative thermogenesis. M2 macrophages could play a role in this context due to their ability to respond to type II cytokines such as **IL-4** and **IL-13** during conditions in which browning is stimulated, such as cold exposure and caloric restriction [19, 35, 49, 59]. Consistent with this notion, depletion of adipose tissue macrophages with clodronate liposomes abolishes browning of subcutaneous inguinal WAT in mice [83], while myeloid-specific **IRE1a** abrogation, which induces M2 macrophages in visceral and subcutaneous fat, promotes norepinephrine signaling and stimulates thermogenic markers in adipocytes [83]. Chronic cold exposure also leads to an increase in the percentage of M2 macrophages in WAT, in part due to macrophage proliferation [35, 84]. Interestingly, this seems to partially require **adiponectin** release by adipocytes, where **T-cadherin** serves as an anchor for tethering of adiponectin to M2 macrophages [84].

M2 macrophages have been proposed to induce browning via local production and secretion of **catecholamines** in response to IL-4 and IL-13 stimulation [17, 51, 59]. However, a study challenged the concept that M2 macrophages produce enough catecholamines themselves to significantly impact browning [33]. While the levels of catecholamines in macrophages may indeed be too low to sustain browning, Luo et al. propose that adrenergic signaling leads to feedforward activation of catecholamine production in adipose tissue macrophages via **CaMKII** activation [85]. In line with this idea, myeloid-specific CaMKIIγ disruption suppresses cold-induced

norepinephrine production and UCP1 expression in subcutaneous fat. Moreover, lack of CaMKII signaling attenuates IL-4/IL-13-mediated catecholamine production [85]. Therefore, while other sources of catecholamines may be more important to elicit browning, these sources may also promote macrophage catecholamine production, which in turn may contribute to the browning process.

Macrophages can also degrade local catecholamine in adipose tissue to inhibit browning. A recent study identified a population of **sympathetic-neuron-associated macrophages (SAM)** in murine adipose tissue expressing **SLC6A2** - a norepinephrine transporter - and monoamine oxidase A (**MAO**)—an enzyme that degrades norepinephrine [34]. By importing and degrading norepinephrine, these macrophages inhibit the ability of the sympathetic nervous system to stimulate browning. Interestingly, the frequency of SAM is increased in obese mice and deletion or inhibition of SLC6A2 in high fat diet-fed mice result in increased adipose tissue thermogenesis and weight loss [34].

While type II cytokines such as **IL-4** and **IL-13** have been shown by several studies to promote browning [17, 36, 49, 51], the role of **IL-10** appears to be more complex than anticipated. Rajbhandari et al. showed that IL-10 signaling represses thermogenic genes and limits thermogenesis [86]. They also found that **IL-10Rα** expression is enriched in adipocytes and increased during obesity and aging [86]. However, another study observed that IL-10 deficiency results in structural abnormalities of BAT mitochondria, cold intolerance and impaired mitochondrial function [87].

IL-33 is a member of the IL-1 family of interleukins which potently drives type II cytokine production by immune cells including ILC2s [36, 69, 88, 89]. IL-33 has an already well documented effect of limiting adipogenesis by means of increasing the thermogenic machinery and mediating the browning of white fat [36]. Beige and brown adipocytes of IL-33 knockout mice fail to express active UCP1, thus compromising uncoupled respiration and thermogenesis [90]. As a consequence, these animals are also more prone to develop obesity under high-fat diet [90]. Conversely, IL-33 promotes energy expenditure [36], and IL-33 treatment or transfer of IL-33-elicited ILC2s is sufficient to induce UCP1 in adipocytes [35, 36]. Notably, helminth infection protects against high fat diet-induced obesity and stimulate a white to beige adipocyte conversion through an IL-33/ILC2-dependent alternative activation of macrophages as a result of type II cytokine release [91]. Indeed, ILC2s express GATA3 and are able to produce type II cytokines in response to IL-33 [36, 51, 92]. However the requirement of type II cytokines and alternative activation of macrophages for IL-33-induced browning is still controversial. For example, while [35] found that IL-33 does not promote browning in IL4R null mice, another group found this receptor is actually dispensable for the IL-33 effect in adipose tissue [36]. In fact, the role of IL-33 in adipose tissue is at the very center of a debate that attempts to accommodate different types of cytokines, immune cells and the browning process within the same mechanistic framework. To make the matter even more complicated, new molecules and modes of intercellular communication are being discovered and seem to play a key role in the process. For instance, Brestoff et al. 2015 propose that the IL-33/ILC2 axis elicits browning of WAT via the production of an enkephalin

peptide named **methionine-enkephalin (Met-Enk),** which binds to opioid receptors on the plasma membrane of beige precursor cells and induces UCP1 [36].

The role of pro-inflammatory cytokines in the browning process may also differ depending on the condition. While **IL-1β** and **TNF-α** have been extensively reported to limit thermogenesis and desensitize β-adrenergic signaling [93, 94], the role of **IL-18** appears to be more complex, given that abrogation of IL-18 or its receptor **(IL18R1)** result in different phenotypes in mice [95]. **IL-6**, on the other hand, may be induced in response to exercise and cancer cachexia, and was shown to promote browning and increase energy expenditure [96, 97]. In addition to being produced by immune cells, IL-6 are expressed at a high level by the exercising muscle [98] and by beige adipocytes [99]. IL-6 was proposed to promote alternative activation of macrophages [48], which in turn leads to BAT activation and beige adipocyte recruitment. Moreover, continuous blockage of the IL-6 receptor during beige adipocyte differentiation results in downregulation of beige adipocyte markers and induction of white adipocyte-like characteristics [99]. IL-6 is considered an important myokine and this may explain why browning occurs under certain conditions upon exercise [98]. Similar to muscle, IL-6 may also act as a BATokine: *i.e.,* when different amounts of BAT are transplanted into the visceral cavity of mice, a dose-dependent improvement of glucose homeostasis is observed, while no such beneficial effects are observed when BAT-transplants are taken from IL-6 knockout mice [100]. In addition, exercise (and cold exposure) induces **meteorin-like protein (Metrnl)** secretion by muscle and WAT [17]. Metrnl is required for long-term cold exposure adaptation and promotes browning, possibly by inducing IL-4 and M2 macrophages [17].

The IL-17 family of cytokines has also been implicated in white to beige adipocyte conversion. A recent study found that **IL-25** acts via its cognate receptor IL-17 receptor B (**IL-17RB**) to promote beige adipogenesis in subcutaneous WAT upon cold or β3-adrenergic stimulation [101]. Mechanistically, IL-25 induces adipose tissue innervation to allow more catecholamine production, and this occurs in part via IL-4 signaling and macrophage activation [101].

4 Novel Mechanisms of Intercellular Communication in Adipose Tissue

Immune cells and adipocytes communicate in ways beyond conventional cytokines. These unconventional ways of immune crosstalk may include signaling lipids [102], growth factor [103] and extracellular miRNAs [104–106]. It remains to be proven whether these molecules are required for immune cell-mediated browning, although the evidence is suggestive.

For example, succinate increases in circulation upon cold exposure and accumulates in BAT and subcutaneous WAT where it acts as a signaling molecule to promote thermogenesis [107]. Succinate also elicits an anti-inflammatory response

in macrophages by binding to the succinate receptor 1 (SUNCR1) [108]. Interestingly, myeloid-specific SUNCR1 knockout mice are prone to metabolic dysfunction and refractory to cold-induced thermogenesis [108]. Together, these results suggest that succinate may act as a signaling molecule in adipocytes and macrophages to sustain adipose tissue browning.

The role of signaling lipids in the browning process still remains elusive. Prostaglandin E2—a classical pro-inflammatory lipid mediator—has been implicated in white to beige adipocyte conversion through the Gs coupled receptor EP4 [109]. Indeed, PGE2-induced EP4 signaling involves cAMP production, which ultimately turn on the thermogenic program. However, the impact of prostaglandins in the browning process is controversial, given that while some studies reported that the upstream and rate-limiting enzyme responsible for the prostaglandins biosynthesis, cyclooxygenase-2 (COX-2), exert a pro-browning effect [110, 111], others proved this enzyme is actually dispensable for browning [112]. Since COXs are the very upstream enzyme on prostaglandin biosynthesis, it is likely that the simultaneous modulation of the other prostaglandins may mask PGE2 thermogenic effects, which may explain the discrepancies between different studies. On the other hand, the inhibition of the microsomal PGE synthase-1 (mPGES-1), a COX-2 downstream terminal synthase responsible for the biosynthesis of PGE2 [109], caused downregulation of browning determination markers in a cell culture setting. In vivo studies with conditional deletion of mPGES-1 in adipocytes and/or in macrophages are required in order to address the questions of whether the pro-thermogenic and pro-browning effects attributed to PGE2 are physiologically relevant and which is the actual source of these lipids under thermal stress or noradrenergic stimulus.

Finally, adipose tissue macrophages [104] and adipocytes [113] may secrete miRNAs in extracellular vesicles, and these miRNAs can be taken up by cells where they control gene expression [24]. Some of these miRNAs have the potential to control the browning process. For example, miR-34a has been shown to inhibit browning [114], while adipocyte-derived miR-34a-containing extracellular vesicles suppress alternative activation of macrophages [106]. Whether this mechanism is required for browning inhibition is unknown.

5 Conclusions

Although a vast list of studies has attributed functions for immune cells and their secreted molecules in the browning process, it appears that we are barely scratching the surface of this matter. Many studies render conflicting results and exactly how immune cells affect adipocytes remains poorly elucidated. Perhaps one of the most consistent observations in the field is the role of IL-33 and ILC2s in beige adipogenesis, but it is still not clear how they influence beige cells, whether through direct signaling or via M2 macrophages. Indeed, another burning issue in the field is the role of M2 macrophages and type II cytokines in the browning process. Whereas a substantial body of evidence supports this relationship, a publication challenged the

relevance of this mechanism to sustain thermogenesis in vivo [33]. These contradictory results may be a consequence of differences in husbandry, microbiota, genetic background, tools or simply the fact that the issue is too complex to be studied by reductionist approaches such as most applied. We believe that more studies are required to establish the exact role of the immune system in the conversion of white adipocytes to beige adipocytes and in BAT thermogenic activation, particularly studies that approach the complex nature of the issue. We are convinced that immune cells within the adipose tissue are absolutely necessary for metabolic homeostasis, and that changes in immune cell function inevitably impacts adipocyte metabolism. How the different cells and molecules participate in this process is an exciting venue for current and future research.

Acknowledgements A.L.R., L.O.L., and M.A.M. are funded by Fundação de Amparo à Pesquisa do Estado de São Paulo (2017/01184-9, 2017/07975-8, 2016/12294-7 and 2017/08264-8). T.J.S. is supported by the German Research Foundation (DFG; SCHU 2445/5-1 and SCHU 2445/6-2), the German Ministry of Education and Research (BMBF) and the State of Brandenburg (DZD grant # 82DZD00302 and FKZ 82DZD0038G).

References

1. Polyzos SA, Kountouras J, Mantzoros CS (2019) Obesity and nonalcoholic fatty liver disease: From pathophysiology to therapeutics. Metabolism 92:82–97
2. Lu FB et al (2018) The relationship between obesity and the severity of non-alcoholic fatty liver disease: systematic review and meta-analysis. Expert Rev Gastroenterol Hepatol 12(5):491–502
3. Luo L, Liu M (2016) Adipose tissue in control of metabolism. J Endocrinol 231(3):R77–R99
4. Arner P, Langin D (2014) Lipolysis in lipid turnover, cancer cachexia, and obesity-induced insulin resistance. Trends Endocrinol Metab 25(5):255–262
5. Zhang Y et al (1994) Positional cloning of the mouse obese gene and its human homologue. Nature 372(6505):425–432
6. Halaas JL et al (1995) Weight-reducing effects of the plasma protein encoded by the obese gene. Science 269(5223):543–546
7. Hotamisligil GS, Shargill NS, Spiegelman BM (1993) Adipose expression of tumor necrosis factor-alpha: direct role in obesity-linked insulin resistance. Science 259(5091):87–91
8. Hotamisligil GS et al (1995) Increased adipose tissue expression of tumor necrosis factor-alpha in human obesity and insulin resistance. J Clin Invest 95(5):2409–2415
9. Hotamisligil GS et al (1996) IRS-1-mediated inhibition of insulin receptor tyrosine kinase activity in TNF-alpha- and obesity-induced insulin resistance. Science 271(5249):665–668
10. Weisberg SP et al (2003) Obesity is associated with macrophage accumulation in adipose tissue. J Clin Invest 112(12):1796–1808
11. Bapat SP et al (2015) Depletion of fat-resident Treg cells prevents age-associated insulin resistance. Nature 528(7580):137–141
12. Catrysse L, van Loo G (2018) Adipose tissue macrophages and their polarization in health and obesity. Cell Immunol 330:114–119
13. Lee BC et al (2016) Adipose Natural Killer Cells Regulate Adipose Tissue Macrophages to Promote Insulin Resistance in Obesity. Cell Metab 23(4):685–698
14. Moraes-Vieira PM et al (2014) RBP4 activates antigen-presenting cells, leading to adipose tissue inflammation and systemic insulin resistance. Cell Metab 19(3):512–526

15. Schipper HS et al (2012) Natural killer T cells in adipose tissue prevent insulin resistance. J Clin Invest 122(9):3343–3354
16. Wu D et al (2011) Eosinophils sustain adipose alternatively activated macrophages associated with glucose homeostasis. Science 332(6026):243–247
17. Rao RR et al (2014) Meteorin-like is a hormone that regulates immune-adipose interactions to increase beige fat thermogenesis. Cell 157(6):1279–1291
18. Chevalier C et al (2015) Gut Microbiota Orchestrates Energy Homeostasis during Cold. Cell 163(6):1360–1374
19. Fabbiano S et al (2016) Caloric Restriction Leads to Browning of White Adipose Tissue through Type 2 Immune Signaling. Cell Metab 24(3):434–446
20. Townsend KL, Tseng YH (2014) Brown fat fuel utilization and thermogenesis. Trends Endocrinol Metab 25(4):168–177
21. Lowell BB, Bachman ES (2003) Beta-Adrenergic receptors, diet-induced thermogenesis, and obesity. J Biol Chem 278(32):29385–29388
22. Stanford KI et al (2018) 12,13-diHOME: An Exercise-Induced Lipokine that Increases Skeletal Muscle Fatty Acid Uptake. Cell Metab 27(6):1357
23. Leiria, L.O., et al., *12-Lipoxygenase Regulates Cold Adaptation and Glucose Metabolism by Producing the Omega-3 Lipid 12-HEPE from Brown Fat.* Cell Metab, 2019.
24. Mori, M.A., et al., *Extracellular miRNAs: From Biomarkers to Mediators of Physiology and Disease.* Cell Metab, 2019.
25. Young P, Arch JR, Ashwell M (1984) Brown adipose tissue in the parametrial fat pad of the mouse. FEBS Lett 167(1):10–14
26. Schulz TJ et al (2011) Identification of inducible brown adipocyte progenitors residing in skeletal muscle and white fat. Proc Natl Acad Sci U S A 108(1):143–148
27. Long JZ et al (2014) A smooth muscle-like origin for beige adipocytes. Cell Metab 19(5):810–820
28. Ikeda K, Maretich P, Kajimura S (2018) The Common and Distinct Features of Brown and Beige Adipocytes. Trends Endocrinol Metab 29(3):191–200
29. Cypess AM et al (2009) Identification and importance of brown adipose tissue in adult humans. N Engl J Med 360(15):1509–1517
30. van Marken Lichtenbelt WD et al (2009) Cold-activated brown adipose tissue in healthy men. N Engl J Med 360(15):1500–1508
31. Virtanen KA et al (2009) Functional brown adipose tissue in healthy adults. N Engl J Med 360(15):1518–1525
32. Nguyen, M.T., et al., *Regulation of chemokine and chemokine receptor expression by PPARgamma in adipocytes and macrophages.* PLoS One, 2012. **7**(4): p. e34976.
33. Fischer K et al (2017) Alternatively activated macrophages do not synthesize catecholamines or contribute to adipose tissue adaptive thermogenesis. Nat Med 23(5):623–630
34. Pirzgalska RM et al (2017) Sympathetic neuron-associated macrophages contribute to obesity by importing and metabolizing norepinephrine. Nat Med 23(11):1309–1318
35. Lee MW et al (2015) Activated type 2 innate lymphoid cells regulate beige fat biogenesis. Cell 160(1–2):74–87
36. Brestoff JR et al (2015) Group 2 innate lymphoid cells promote beiging of white adipose tissue and limit obesity. Nature 519(7542):242–246
37. Kohlgruber AC et al (2018) gammadelta T cells producing interleukin-17A regulate adipose regulatory T cell homeostasis and thermogenesis. Nat Immunol 19(5):464–474
38. Sanchez-Gurmaches J, Hung CM, Guertin DA (2016) Emerging Complexities in Adipocyte Origins and Identity. Trends Cell Biol 26(5):313–326
39. Zwick RK et al (2018) Anatomical, Physiological, and Functional Diversity of Adipose Tissue. Cell Metab 27(1):68–83
40. Meza-Perez S, Randall TD (2017) Immunological Functions of the Omentum. Trends Immunol 38(7):526–536
41. Abate N et al (1995) Relationships of generalized and regional adiposity to insulin sensitivity in men. J Clin Invest 96(1):88–98

42. Park KS et al (1991) Intra-abdominal fat is associated with decreased insulin sensitivity in healthy young men. Metabolism 40(6):600–603
43. Yamashita S et al (1996) Insulin resistance and body fat distribution. Diabetes Care 19(3):287–291
44. Tchkonia T et al (2005) Abundance of two human preadipocyte subtypes with distinct capacities for replication, adipogenesis, and apoptosis varies among fat depots. Am J Physiol Endocrinol Metab 288(1):E267–E277
45. Alkhouri N et al (2010) Adipocyte apoptosis, a link between obesity, insulin resistance, and hepatic steatosis. J Biol Chem 285(5):3428–3438
46. Sell H, Habich C, Eckel J (2012) Adaptive immunity in obesity and insulin resistance. Nat Rev Endocrinol 8(12):709–716
47. Lumeng CN et al (2011) Aging is associated with an increase in T cells and inflammatory macrophages in visceral adipose tissue. J Immunol 187(12):6208–6216
48. Mauer J et al (2014) Signaling by IL-6 promotes alternative activation of macrophages to limit endotoxemia and obesity-associated resistance to insulin. Nat Immunol 15(5):423–430
49. Qiu Y et al (2014) Eosinophils and type 2 cytokine signaling in macrophages orchestrate development of functional beige fat. Cell 157(6):1292–1308
50. Lizcano F et al (2017) Human ADMC-Derived Adipocyte Thermogenic Capacity Is Regulated by IL-4 Receptor. Stem Cells Int 2017:2767916
51. Molofsky AB et al (2013) Innate lymphoid type 2 cells sustain visceral adipose tissue eosinophils and alternatively activated macrophages. J Exp Med 210(3):535–549
52. Fitzgibbons TP et al (2011) Similarity of mouse perivascular and brown adipose tissues and their resistance to diet-induced inflammation. Am J Physiol Heart Circ Physiol 301(4):H1425–H1437
53. Herrero L et al (2010) Inflammation and adipose tissue macrophages in lipodystrophic mice. Proc Natl Acad Sci U S A 107(1):240–245
54. Bae J et al (2014) Activation of pattern recognition receptors in brown adipocytes induces inflammation and suppresses uncoupling protein 1 expression and mitochondrial respiration. Am J Physiol Cell Physiol 306(10):C918–C930
55. McGregor RA et al (2013) Time-course microarrays reveal modulation of developmental, lipid metabolism and immune gene networks in intrascapular brown adipose tissue during the development of diet-induced obesity. Int J Obes (Lond) 37(12):1524–1531
56. Sakamoto T et al (2013) Inflammation induced by RAW macrophages suppresses UCP1 mRNA induction via ERK activation in 10T1/2 adipocytes. Am J Physiol Cell Physiol 304(8):C729–C738
57. Sakamoto T et al (2016) Macrophage infiltration into obese adipose tissues suppresses the induction of UCP1 level in mice. Am J Physiol Endocrinol Metab 310(8):E676–E687
58. Ricardo-Gonzalez RR et al (2010) IL-4/STAT6 immune axis regulates peripheral nutrient metabolism and insulin sensitivity. Proc Natl Acad Sci U S A 107(52):22617–22622
59. Nguyen KD et al (2011) Alternatively activated macrophages produce catecholamines to sustain adaptive thermogenesis. Nature 480(7375):104–108
60. Cereijo, R., et al., *CXCL14, a Brown Adipokine that Mediates Brown-Fat-to-Macrophage Communication in Thermogenic Adaptation.* Cell Metab, 2018. **28**(5): p. 750–763 e6.
61. Wolf Y et al (2017) Brown-adipose-tissue macrophages control tissue innervation and homeostatic energy expenditure. Nat Immunol 18(6):665–674
62. Talukdar S et al (2012) Neutrophils mediate insulin resistance in mice fed a high-fat diet through secreted elastase. Nat Med 18(9):1407–1412
63. Liu J et al (2009) Genetic deficiency and pharmacological stabilization of mast cells reduce diet-induced obesity and diabetes in mice. Nat Med 15(8):940–945
64. Zhang, X., et al., *Functional Inactivation of Mast Cells Enhances Subcutaneous Adipose Tissue Browning in Mice.* Cell Rep, 2019. **28**(3): p. 792–803 e4.
65. Finlin BS et al (2017) Mast Cells Promote Seasonal White Adipose Beiging in Humans. Diabetes 66(5):1237–1246

66. Winer DA et al (2014) B Lymphocytes in obesity-related adipose tissue inflammation and insulin resistance. Cell Mol Life Sci 71(6):1033–1043
67. Nishimura S et al (2009) CD8+ effector T cells contribute to macrophage recruitment and adipose tissue inflammation in obesity. Nat Med 15(8):914–920
68. Feuerer M et al (2009) Lean, but not obese, fat is enriched for a unique population of regulatory T cells that affect metabolic parameters. Nat Med 15(8):930–939
69. Vasanthakumar A et al (2015) The transcriptional regulators IRF4, BATF and IL-33 orchestrate development and maintenance of adipose tissue-resident regulatory T cells. Nat Immunol 16(3):276–285
70. Odegaard JI et al (2017) Perinatal Licensing of Thermogenesis by IL-33 and ST2. Cell 171(7):1707
71. Eller K et al (2011) Potential role of regulatory T cells in reversing obesity-linked insulin resistance and diabetic nephropathy. Diabetes 60(11):2954–2962
72. Chen X, Wu Y, Wang L (2013) Fat-resident Tregs: an emerging guard protecting from obesity-associated metabolic disorders. Obes Rev 14(7):568–578
73. Medrikova, D., et al., *Brown adipose tissue harbors a distinct sub-population of regulatory T cells.* PLoS One, 2015. **10**(2): p. e0118534.
74. Kalin, S., et al., *A Stat6/Pten Axis Links Regulatory T Cells with Adipose Tissue Function.* Cell Metab, 2017. **26**(3): p. 475–492 e7.
75. DeFuria J et al (2013) B cells promote inflammation in obesity and type 2 diabetes through regulation of T-cell function and an inflammatory cytokine profile. Proc Natl Acad Sci U S A 110(13):5133–5138
76. Peterson KR, Flaherty DK, Hasty AH (2017) Obesity Alters B Cell and Macrophage Populations in Brown Adipose Tissue. Obesity (Silver Spring) 25(11):1881–1884
77. Kin NW, Sanders VM (2006) It takes nerve to tell T and B cells what to do. J Leukoc Biol 79(6):1093–1104
78. Saze Z et al (2013) Adenosine production by human B cells and B cell-mediated suppression of activated T cells. Blood 122(1):9–18
79. Lynch L et al (2012) Adipose tissue invariant NKT cells protect against diet-induced obesity and metabolic disorder through regulatory cytokine production. Immunity 37(3):574–587
80. Lynch L et al (2016) iNKT Cells Induce FGF21 for Thermogenesis and Are Required for Maximal Weight Loss in GLP1 Therapy. Cell Metab 24(3):510–519
81. Molofsky AB, Savage AK, Locksley RM (2015) Interleukin-33 in Tissue Homeostasis, Injury, and Inflammation. Immunity 42(6):1005–1019
82. Mahlakoiv, T., et al., *Stromal cells maintain immune cell homeostasis in adipose tissue via production of interleukin-33.* Sci Immunol, 2019. **4**(35).
83. Shan B et al (2017) The metabolic ER stress sensor IRE1alpha suppresses alternative activation of macrophages and impairs energy expenditure in obesity. Nat Immunol 18(5):519–529
84. Hui X et al (2015) Adiponectin Enhances Cold-Induced Browning of Subcutaneous Adipose Tissue via Promoting M2 Macrophage Proliferation. Cell Metab 22(2):279–290
85. Luo Y et al (2017) Myeloid adrenergic signaling via CaMKII forms a feedforward loop of catecholamine biosynthesis. J Mol Cell Biol 9(5):422–434
86. Rajbhandari, P., et al., *IL-10 Signaling Remodels Adipose Chromatin Architecture to Limit Thermogenesis and Energy Expenditure.* Cell, 2018. **172**(1–2): p. 218–233 e17.
87. de-Lima-Junior, J.C., et al., *Abnormal brown adipose tissue mitochondrial structure and function in IL10 deficiency.* EBioMedicine, 2019. **39**: p. 436–447.
88. Ding X et al (2016) IL-33-driven ILC2/eosinophil axis in fat is induced by sympathetic tone and suppressed by obesity. J Endocrinol 231(1):35–48
89. Cayrol C, Girard JP (2018) Interleukin-33 (IL-33): A nuclear cytokine from the IL-1 family. Immunol Rev 281(1):154–168
90. Odegaard JI et al (2016) Perinatal Licensing of Thermogenesis by IL-33 and ST2. Cell 166(4):841–854
91. Su CW et al (2018) Helminth infection protects against high fat diet-induced obesity via induction of alternatively activated macrophages. Sci Rep 8(1):4607

92. Moro K et al (2010) Innate production of T(H)2 cytokines by adipose tissue-associated c-Kit(+)Sca-1(+) lymphoid cells. Nature 463(7280):540–544
93. Goto T et al (2016) Proinflammatory cytokine interleukin-1beta suppresses cold-induced thermogenesis in adipocytes. Cytokine 77:107–114
94. Nisoli E et al (2000) Tumor necrosis factor alpha mediates apoptosis of brown adipocytes and defective brown adipocyte function in obesity. Proc Natl Acad Sci U S A 97(14):8033–8038
95. Pazos P et al (2015) Divergent responses to thermogenic stimuli in BAT and subcutaneous adipose tissue from interleukin 18 and interleukin 18 receptor 1-deficient mice. Sci Rep 5:17977
96. Knudsen, J.G., et al., *Role of IL-6 in exercise training- and cold-induced UCP1 expression in subcutaneous white adipose tissue.* PLoS One, 2014. **9**(1): p. e84910.
97. Petruzzelli M et al (2014) A switch from white to brown fat increases energy expenditure in cancer-associated cachexia. Cell Metab 20(3):433–447
98. Wedell-Neergaard, A.S., et al., *Exercise-Induced Changes in Visceral Adipose Tissue Mass Are Regulated by IL-6 Signaling: A Randomized Controlled Trial.* Cell Metab, 2019. **29**(4): p. 844–855 e3.
99. Kristof E et al (2019) Interleukin-6 released from differentiating human beige adipocytes improves browning. Exp Cell Res 377(1–2):47–55
100. Stanford KI et al (2013) Brown adipose tissue regulates glucose homeostasis and insulin sensitivity. J Clin Invest 123(1):215–223
101. https://doi.org/10.1371/journal.pbio.3001348
102. Klein-Wieringa IR et al (2013) Adipocytes modulate the phenotype of human macrophages through secreted lipids. J Immunol 191(3):1356–1363
103. Schulz TJ et al (2016) Loss of BMP receptor type 1A in murine adipose tissue attenuates age-related onset of insulin resistance. Diabetologia 59(8):1769–1777
104. Ying, W., et al., *Adipose Tissue Macrophage-Derived Exosomal miRNAs Can Modulate In Vivo and In Vitro Insulin Sensitivity.* Cell, 2017. **171**(2): p. 372–384 e12.
105. Liu T et al (2019) Adipose tissue macrophage-derived exosomal miR-29a regulates obesity-associated insulin resistance. Biochem Biophys Res Commun 515(2):352–358
106. Pan Y et al (2019) Adipocyte-secreted exosomal microRNA-34a inhibits M2 macrophage polarization to promote obesity-induced adipose inflammation. J Clin Invest 129(2):834–849
107. Mills EL et al (2018) Accumulation of succinate controls activation of adipose tissue thermogenesis. Nature 560(7716):102–106
108. Keiran N et al (2019) SUCNR1 controls an anti-inflammatory program in macrophages to regulate the metabolic response to obesity. Nat Immunol 20(5):581–592
109. Garcia-Alonso V et al (2013) Coordinate functional regulation between microsomal prostaglandin E synthase-1 (mPGES-1) and peroxisome proliferator-activated receptor gamma (PPARgamma) in the conversion of white-to-brown adipocytes. J Biol Chem 288(39):28230–28242
110. Madsen, L., et al., *UCP1 induction during recruitment of brown adipocytes in white adipose tissue is dependent on cyclooxygenase activity.* PLoS One, 2010. **5**(6): p. e11391.
111. Vegiopoulos A et al (2010) Cyclooxygenase-2 controls energy homeostasis in mice by de novo recruitment of brown adipocytes. Science 328(5982):1158–1161
112. Paschos GK et al (2018) Cold-Induced Browning of Inguinal White Adipose Tissue Is Independent of Adipose Tissue Cyclooxygenase-2. Cell Rep 24(4):809–814
113. Thomou T et al (2017) Adipose-derived circulating miRNAs regulate gene expression in other tissues. Nature 542(7642):450–455
114. Fu T et al (2014) MicroRNA 34a inhibits beige and brown fat formation in obesity in part by suppressing adipocyte fibroblast growth factor 21 signaling and SIRT1 function. Mol Cell Biol 34(22):4130–4142. https://doi.org/10.1126/sciadv.abc6250

Physical Exercise and Metabolic Reprogramming

H. P. Batatinha, F. S. Lira, K. Kruger, and J. C. Rosa Neto

1 Metabolic Reprograming with Aerobic and Resistance Exercise

Chronic exercise improves cardiovascular health and increases skeletal muscle hypertrophy, promoting several adaptations. In the liver, exercise reduces inflammation, resulting in improved insulin sensitivity with superior, fine-tuned endogenous glucose production; in terms of brain health, the benefits include neuroprotection and neurogenesis. This improvement promoted by regular exercise is due to the adaptations of the whole body to exercise-induced stress. These adaptations can be defined as "reprograming."

Metabolic reprogram is a cellular adaptation in the internal machinery via regulation of transcription factors, epigenetic alterations, mRNA expression, enzyme activity, and storage of energy substrates. This exercise-induced reprograming depends of the type (aerobic, strength, or concurrent exercise session), volume (short or long duration), intensity (high, moderate, or low), recovery during the exercise session (passive, active, short, or long), and duration of the exercise program (2 week, 6 week, 12 week, 24 week, 48 week, 1 year, 5 year, or 10 year).

Here, we will focus on metabolic reprograming in the skeletal muscles, adipose tissues, and immune cells after aerobic and strength training. By all of regular exercise training, strength and endurance are the most studied. Strength training improves s capacity of keletal muscle to contract, while aerobic training improves oxygen

H. P. Batatinha · J. C. Rosa Neto (✉)
Department of Cell and Developmental Biology, University of São Paulo, São Paulo, Brazil
e-mail: jrosaneto@usp.br

F. S. Lira
Department of Physical Education, Universidade Estadual Paulista, Presidente Prudente, Brazil

K. Kruger
Department of Exercise Physiology and Sports Therapy, Justus-Liebig-University Giessen, Giessen, Germany

N. O. S. Camara et al. (eds.), *Essential Aspects of Immunometabolism in Health and Disease*, https://doi.org/10.1007/978-3-030-86684-6_12

Uptake, from the blood, and delivery to the muscle. Thus, both strength and aerobic exercise training are prescribed to improve cardiovascular fitness, force production, and body composition. Independent of the exercise type, actually, exercise is considered an excellent non-pharmacological method of preventing and treating several chronic inflammatory diseases [1]. In contrast, a sedentary lifestyle is an independent risk factor in condition called the diseasome of physical inactivity [2].

Aerobic exercise, classically adopted for cardiorespiratory fitness and control of body fat, is usually performed as a moderate-intensity (50–70% VO_2max) continuous training (MICT) (running, bike, indoor or outdoor). Recent studies have suggested that aerobic exercise can exert the same or similar adaptation when performed as high-intensity (90–120% VO_2max) interval training (HIIT) in the same modalities and can be adopted in various forms. Some studies have shown that HIIT can positively impact the neuroendocrine axis, such as the leptin levels, and improve lipid metabolism in overweight/obese and lean subjects [3–6].

In order to obtain a deeper understanding about how exercise training can induce metabolic reprograming, the acute effects of each exercise session need to be examined in detail.

Figure 1 shows main acute and chronic effects of aerobic exercise performed at different intensities on the skeletal muscle and adipose tissue.

A substantial proportion of the alterations caused by a single bout of aerobic exercise is related to neuro-endocrine axis activation. Acute exercise enhances several hormones, especially those related to lipolysis in the adipose tissue and glycogenolysis in the skeletal muscle and liver (cortisol and adrenaline) that increase the availability of energy substrates (Non-esterified fatty acids (NEFA) and glucose) for muscle workload. With aerobic exercise both, carbohydrates and fat can be utilized for oxidation, and the metabolic changes are dependent on the exercise intensity and training status of the subjects.

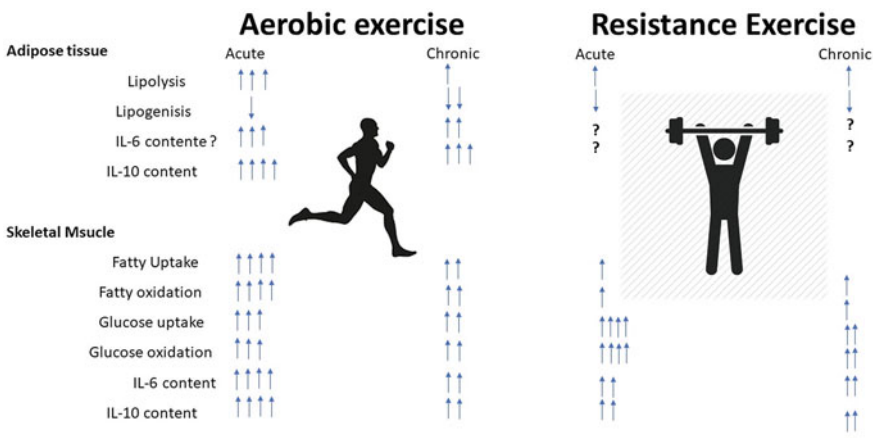


Fig. 1 Different roles of acute and chronic exercise in adipose tissue and skeletal muscle

The crossover point that leads to better oxidation of carbohydrate that the fatty acid in proportion, it is approximately in 65% of VO_2max. However, the point of this bypass characterized by the predominance of carbohydrates as the major fuel for oxidation is dependent on the training levels. Sedentary subjects have a lower capacity of oxidation with exercise performed at intensities >45% VO_2max, while well-trained athletes showed this threshold at intensities of approximately 75% VO_2max. This is because fatty acid mobilization, transport, and oxidation are not easy, and athletes have superior adaptation to lipid metabolism [7, 8].

Thus, it is necessary to coordinate the mobilization of fatty acids from the adipose tissue, transport in the plasma, and fatty acid uptake by the skeletal muscle; once FA is inside the skeletal muscle, it has to be transported to the mitochondria and finally oxidized for ATP production [8]. The main adaptations involved in lipids metabolic reprograming are associated with increased delivery of fatty acids by the subcutaneous adipose tissue (SAT) and increased fatty acid uptake by the skeletal muscle [9]. The blood flow in the SAT increases 2–3 folds in endurance athletes [9]. This is an important step in the delivery of fatty acids from the adipose tissue to the skeletal muscle; however, albumin is the other limiting factor for fatty acid transport. Moreover, fatty acid transportation from circulation to inside the skeletal muscle is carried out by CD36 transporter in the plasma membrane of myocytes. Acute exercise and training induce increase in the CD36 in the sarcolemma [10–12].

Thus, metabolic reprograming can be observed when aerobic exercise is maintained for a long time all machinery is organizing for better efficiency for mobilization and oxidation of FA.

Well-trained individuals tend to store lipids in the skeletal muscle, similar to that found in diabetic patients; however, with exercise, fat accumulation does not harm cellular functioning, as is observed in metabolic diseases [13, 14].

Lipid storage in myofibers is mediated by perilipins, the perilipin-2, perilipin-3, and perilipin-5, the most common perilipins founded in lipid droplets (LD) in the skeletal muscle. An interesting study conducted by Daemmen et al. 2018 showed that the localization and size of the LD was different between diabetic subjects and athletes although the content of the LD was the same. In this sense, the diabetic subjects showed larger LD in type II fyber, and the athletes have smaller LD in type I fiber [15]. Moreover, athletes present lipid deposition near the mitochondria with higher expression of ATGL and CGI-58 [16]. Thus, these adaptations cause high LD turnover, optimize the energy production machinery to supply energy during exercise, and mitigate the lipotoxicity that could be generated by increased fatty acid accumulation [17].

AMP-activated protein kinase (AMPK), MAP kinase p38, calmodulin-dependent protein kinase (CAMKK), PGC-1alpha, and p53 form the axis that induces mitochondrial remodeling and increases electron chain transporters to maximize the oxidative phosphorylation. Increased Ca^{2+} concentration via muscle contraction directly activates CAMKK [18].

In general, both types of aerobic exercise training (MICT and HIIT) promote similar metabolic reprograming in the skeletal muscle and adipose tissue. However,

short-term (<12 weeks) exercise training is insufficient to promote these cellular adaptations, while long-term (>12 weeks) training is able to promote them [4, 19, 20].

It is noteworthy that the same pathways responsible for energy-induced metabolic reprograming also lead to the production of proteins called myokines [21]. Myokines are a group of proteins formed by cytokines, chemokines, and growth factors produced by muscle cells that are released into the circulation and act in distant organs and different cell types [22, 23]. The most studied myokine is IL-6 [24].

IL-6 is a major moderator involved in the regulation of the acute-phase response to injury and infection. The skeletal musclecontraction induces the activation of the mitogen-activated protein kinase and Janus kinase (JAK)-signal transducers and activators of transcription cascade phosphorylation, culminating with IL-6 transcription in skeletal muscle [25]. The IL-6 produced and secreted by the skeletal muscle during contraction plays an important role in metabolism. Moreover, it induces the translocation of GLUT4, in the muscle, increasing glycogen synthesis and insulin sensitivity in the central and peripheral organs as well as augments lipid oxidation in the skeletal muscle to deliver fuel to source the skeletal muscle during exercise [22].

Prolonged and intense running on a treadmill at 70% $VO_{2\,m}$ax until exhaustion increased the muscle plasma levels for IL-6, and the post-run muscle glycogen concentrations were negatively correlated with modifications in muscle IL-6. Cabral-Santos et al. [26] found that during acute high-intensity interval exercise, there was a volume-dependent increase in the IL-6 levels.

IL-6 modifies the anti-inflammatory responses once it can induce anti-inflammatory cytokines production, such as IL-10 and IL-1ra [27]. IL-10 is essentiall in the anti-inflammatory response. It promotes the preservation of IkB, inhibiting the nuclear transcription factor kappa B (NF-kB) signaling, the main transcription factor of TNF-α [27]—a cytokine with a pro-inflammatory character that regulates insulin sensitivity and induces lipolysis. Cabral-Santos et al. [28] found significantly increased IL-6 and IL-10 levels after HIIE (1:1-min at vVO2 max, 5 km run), indicating a crucial role of HIIE in preventing a persistent inflammatory tendency.

Strength training also promotes metabolic reprograming; however, the cellular mechanism and the metabolic pathways are different because strength (resistance) training induces anabolic pathways that are contrary to the catabolic pathways induced by aerobic training. The main cellular response is the increase in the cross sectional area by the induction of the hypertrophy pathway controlled by the mTOR-p70S6 axis.

Different forms can activate the mTOR-P70S6. In physiological context, the insulin and growth factors (in special IGF-1) lead to activation of mTOR toward the activation of PI3K-Akt, this pathway is upstream to mTOR. It was previously believed that the activation of exercise-induced hypertrophy was caused by increase in the anabolic hormones and the PI3k pathway; however, simulation with IGF-1 was insufficient to mimic the hypertrophy induced by mechanical induction. Currently, the principal hypothesis is that the mechanical overload activates the dissociation of TSC2 (an inhibitor of mTOR-Rheb complex) in the lysosomes assembly and allows downstream signaling with the activation of p70. However, it is unclear how TSC2

phosphorylation and dissociation of mTOR-rheb complex occur, and more studies are necessary to identify the mechanism [29, 30].

The effects of resistance training are dependent on the size of the muscle skeletal recruited during exercise; for example, training of the biceps brachial induces local adaptation; however, this effect is not endocrine, and leg exercise training induces a huge endocrine response, with increase in the production and release of myokines. The fall in the serum myokine concentration is more rapid than that with endurance training.

Intensity and recovery intervals are the fundamental factors for changes in the cytokine response, main IL-6 alterations in resistance training [31, 32]. These factors influence the number of repetitions per- formed in subsequent set. In this sense, Izquierdo et al. [33] verified that the amount of metabolic demand or the fatigue experienced during the strength exercise session affects the hormonal and cytokine response patterns.

Myokines, specifically IL-6, play a role in skeletal muscle tissue regeneration after exercise as well as the recruitment of neutrophils, monocytes, and lymphocytes that phagocytize debris resources. The IL-6 aids the activation, differentiation and proliferation of satellite cells, which contribute to the formation of new myonuclei. IL-6 act on mTORC1 signaling and protein synthesis in the cultured myotubes [34]. It was observed that IL-6 activates gp130-Akt and mTORC1 pathways, inducing protein synthesis, suggesting that the IL-6 response to strength exercise potentiates muscle hypertrophy [34].

A combination of strength and aerobic exercises in the same session can be called concurrent exercise. Acute and chronic concurrent sessions promote alterations in the IL-6 and TNF-α responses that can be associated with an acute decline in the strength performance observed when high-intensity interval aerobic exercise is performed before strength exercise. This response was related a possible role of TNF-α and IL-6 as a trigger to restore the energy demand by providing substrates to help maintain contractile activity in skeletal muscle [35]. Long-term (12 weeks) practice for concurrent training leads to improved cardiorespiratory fitness and maximal strength accompanied with an increased cytokines response, leading to an anti-inflammatory response after an acute session of concurrent exercise. However, the effects of concurrent training in cellular reprograming remain unclear [36, 37].

The high-energy demand of exercise, especially in the case of long-duration and high-intensity exercise, such as that performed by long-distance runners, aquatic marathoners, and triathletes, is associated with stress hormonal response (increased catecholamines and cortisol) causing deep alterations in the immune cells.

2 Exercise and Immune System

The relationship of exercise and the immune system was first discussed during the late 70s and early 80s when some researchers observed that a single bolt of exercise (mainly aerobic) could cause an acute increase in the number of leucocytes in

the circulation, with the levels returning to pre-exercise levels within some hours after activity cessation [38]. At this time, researches had already established the potential role of some hormones in this relationship and proposed that cortisol could mediate the re-sequestration of the immune cells. Several years thereafter, a research group from Denmark showed that this exercise-induced leukocytosis was, in part, catecholamine-dependent once catecholamines were released during exercise and the leucocytes expressed β2-adrenergic receptors [39].

Neutrophils are released in the highest amount after exercise, and their increased levels are maintained in the circulation for up to 6 h after exercise cessation. Complete recovery is generally achieved within 24 h [40]. Most of these cells are immature, less differentiated, and precursor neutrophils; thus, despite increased numbers, the function is decreased, especially after high-intensity and prolonged exercise (>2 h).

Monocytes also increase in response to high-intensity and long-duration exercise. In case of very prolonged exercise sessions, monocytosis is delayed by 1–2 h. In most cases, monocytes return to the rest levels 6 h after exercise cessation. Some studies also report a decrease in the function of these cells in response to exhaustive exercise [41].

Lymphocytes play a different role. Immediately after prolonged and/or high-intensity exercise, lymphocytes are also increased in the circulation; however, the number of lymphocytes decreases below the pre-exercise values within 30 min, returning to rest levels by up to 6 h after exercise cessation [41]. This lymphopenia is more prominent in the lymphocyte subtypes with potent effector functions (e.g., natural killer (NK) cells, γδT cells, and $CD8^+$T cells). Moreover, T cells proliferation, NK cell cytotoxicity, and IgA salivary concentration are reportedly inhibited after a bout of very prolonged exercise [42].

The decrease in the function of immune cells in response to exhaustive exercise has been well documented in the literature. Two decades previously, the "open window" hypothesis was postulated proposing that up to 72 h after a high-intensity and prolonged exercise season, the host body would be more susceptible to viral infections because of immunodepression [43]. It could be due to a high concentration of cortisol in the blood in response to exercise stress and the decrease of substrates (glucose and glutamine) availability to the immune cells. This classic paradigm in the field of exercise immunology is still being understood with several contradictory results.

However, some studies, have reported a positive correlation between exhaustive exercise and upper respiratory tract infection (URTI). In 1994, Nieman [44] proposed a "J-shaped" curve relationship between the exercise workload and URTI risk. It indicates that sedentary individuals are at a higher risk of URTI than those who exercised moderately; however, those performing heavy exercise may have a higher risk than who led a sedentary lifestyle.

This "J-shaped" curve was challenged in many studies where they failed to find this relationship. Thus, an "S-shaped" curve was proposed [45], suggesting that very elite athletes are better adapted to the demands of their training and are less susceptible to URTI. It is noteworthy that this population was at a lower risk of URTI and had a better quality of recovery and nutrition, suggesting that exhaustive exercise

by itself may not promote deep immunodepression and increase URTI risk; rather, these consequences were attributable to inappropriate recovery and poor nutrition.

The inability to maintain an exercise workload can be classified as acute fatigue. It may be caused by repeated bouts of intense exercise on the same day and/or during consecutive days without adequate recovery. Athletes who aim to improve their performance are often close to this fatigue and intend to reach an "overreaching" state when performance is enhanced. Continuous intense training without proper balance between training and recovery may surpass "overreaching" and result in an "overtraining syndrome." [46]. At this point, fatigue is persistent, performance is considerably decreased, hormonal status is disturbed, and immune functions are declined. Over-trained individuals present with a decrease in the plasma glutamine concentration and salivary IgA and are at a higher risk of URTI.

High-intensity and prolonged training can cause transient immunodepression because overreaching athletes present reduction in resting neutrophil degranulation, lymphocyte proliferation, and antibody production. However, their immune functions are restored, preventing a rise in the URTI risk. In contrast, over-trained athletes cannot recover from the overreaching stage and frequently experience upper respiratory tract illnesses and chronic decrease in immune cells cytokines and antibody production [46].

Not only exercise workload and immune system, but also nutritional status influences the exercise recovery. During the previous 30 years, scientists have investigated nutritional strategies to prevent exercise-induced immunodepression and its complications.

The energy substrate is strongly related to immune cell function. Immune cells are dependent on the substrate not only for energy generation, but also for the creation of new molecules, via the action of biosynthetic pathways.

Long-distance athletes showed reduction in the glucose and glutamine disposal to the immune cells. The skeletal muscles utilize a high proportion of the available glucose to generate ATP via the glycolytic and oxidative pathways. Furthermore, the carbon skeletons provided by amino acids generated via induction on muscle proteolysis, which is induced by stress hormones (in special cortisol), are delivered to liver to formation of glucose by gluconeogenesis (Kou et al., 2013). Thus, the disposal of glucose and glutamine to the immune cells is reduced. This reduction causes the activation of AMPK that induces anti-inflammatory signature in the immune cells. It is very interesting that metabolic reprograming in immune cells is similar to that in the muscle cells, with an antagonist response between AMPK and mTOR activation.

AMPK has been demonstrated as a key metabolic regulator of T cells, essential for ATP homeostasis; further, it allows lymphocytes metabolic plasticity to adapt to the energy stress. However, AMPK does not play a role in cell effector function. Once activated, AMPK shuts off the anabolic pathways, including mTOR. mTOR complex 1 (mTORC1) is essential for immune cell activation and proliferation; however, its constant activation increases the reactive oxygen species and induces cell senescence. Therefore, the activation of AMPK or mTOR is essential for determining the fate of the immune cells and deciding the activation of the pro-inflammatory or anti-inflammatory profile [47].

It is unclear exactly how exercise induces the reprograming of immune cells; however, the myokines released after exercise, increased hormonal stress (particularly cortisol levels), and substrate available due to the reduction of glucose and specific amino acids in the circulation are responsible for inducing this reprograming [48].

Carbohydrate is the most researched nutrient, and several researches have investigated its supplementation before, during, and after a high-intensity and/or prolonged exercise. Carbohydrate ingestion during prolonged exercise can maintain the blood glucose levels and attenuate the release of stress hormones and exercise-induced cytokines. They also influence recovery by reducing the suppression of TCD4 and TCD8 lymphocytes for 24 h after exercise cessation [49].

Similar results were found when carbohydrate was consumed till up to 15 min before the exercise, preventing immunoendocrine disturbances. However, carbohydrate ingestion only during the recovery period has not shown such effects.

Carbohydrate supplementation is important for the maintenance of immune system functions during exercise and better recovery. Thus, it could be an effective strategy for decreasing the potential cumulative immunodepression over consecutive days of exercise to avoid overtraining. Researchers have found that carbohydrate ingestion during consecutive days of a heavy training regime was able to attenuate plasma cortisol levels and enhance lymphocytes proliferation and function, thus reducing exercise-induced adaptive immune depression [50].

Protein ingestion is also a concern in the immune nutrition area because it is essential for proper immune system function. A high-protein diet (3 g/kg/day) during a period of intense training decreased exercise-induced impairment in lymphocytes trafficking when compared to a carbohydrate-matched normal protein diet [51]. When supplemented for 6 days, during a heavy training period, protein ingestion decreased neutrophil degranulation during the post exercise recovery period [52]. It indicates that protein administration could be an approach to attenuate chronic immunodepression after a long period of intense training; however, it did not appear to have the same acute effect as carbohydrates.

In contrast to the immunodepression caused by high-intensity and prolonged exercise, moderate-intensity single or repeated bouts of exercise appear to exert a contradictory role. Several studies indicate that frequent participation in regular exercise can stimulate the immune system and decrease respiratory infections.

Moderate aerobic exercise appears as an effector-T cells activator, stimulating them to transmigrate to the peripheral tissues where immune surveillance is enhanced by physical stress. Recent data has shown that short-term intense exercise (30 min of cycling) increased virus-specific T cells mobilization, increasing the success of immunotherapy for viral infections. It also mobilized CD8 naïve T cells and high differentiated and EMRA CD8+ subsets [53]**.** Furthermore, moderate exercise may mobilize the senescent phenotype lymphocytes to undergo apoptosis in the tissues allowing "fresh" ones to take their place, thus possibly improving the immune response against infections.

A recent study showed that acute exercise mobilizes angiogenic T cells, facilitating vascular remodeling during recovery after exercise [54]. Hematopoietic stem cells

are also mobilized after aerobic exercise, and this could aid skeletal muscle repair and regeneration for exercise recovery (Kruger et al. 2015). This exercise-induced stem cells mobilization has been studied as an adjuvant therapy for hematopoietic stem cell transplantation.

Neutrophil function is also enhanced by moderate exercise. One hour of cycling at 50% of VO2 max increased the neutrophil oxidative burst and during the early stage of recovery, the bacterial activity of these cells continues to increase up to 1 h after exercise [55]. Monocytes mobilized by exercise might infiltrate the skeletal muscle and differentiate into tissue-resident macrophages facilitating tissue repair and regeneration. Moreover, the expression of pathogen-recognition receptors, such as toll-like receptors, tends to decrease in response to moderate-intensity exercise that could contribute to the anti-inflammatory role of exercise training in metabolic diseases. In general, short bouts of moderate-intensity exercise might enhance cellular immune function [56].

Exercise training has the ability to decrease low-grade chronic inflammation, restore antioxidant mechanisms, and stimulate immune cells response, which may reduce age-related immunosenescence [57]. In a study conducted on Judo masters athletes, it was observed that they senescent naïve and effector memory CD8+ T cells and CD4+ T cells and VO2 max was positively correlated with naïve CD4+ T cells population, indicating that lifelong exercise could positively regulate immune function [58].

In studies on vaccination and exercise, physically active individuals appear to have better response than sedentary individuals. Older adults (62 years old) who performed at least 20 min of aerobic exercise 3–4 times a week showed higher anti-influenza IgG and IgM titers versus sedentary adults 2 weeks after vaccination. The exercise group also exhibited increased peripheral blood mononuclear cells in response to antigen-specific stimulation (Araujo et al. 2015). Moreover, in an animal model, mice who exercised before being exposed to the influenza virus showed lower severity of infection and inflammation in the lungs [59]. In another study, exercise before virus exposure was able to increase the survival rates in mice.

Thus, high-intensity and prolonged exercise without a proper recovery and nutrition can lead to an immunodepression and increase the risk of URTI. However, these finds indicate that exercise is a potent immunomodulator because continuous lower doses in moderate intensity can improve the immune response and decrease inflammation in metabolic diseases (Fig. 2).

3 Exercise for the Treatment and Prevention of Metabolic Disease

The increased prevalence of metabolic disorders and its various features, such as obesity and type 2 diabetes (T2D), represents an enormous challenge for health systems worldwide. It affects more than 20% of the general population in most

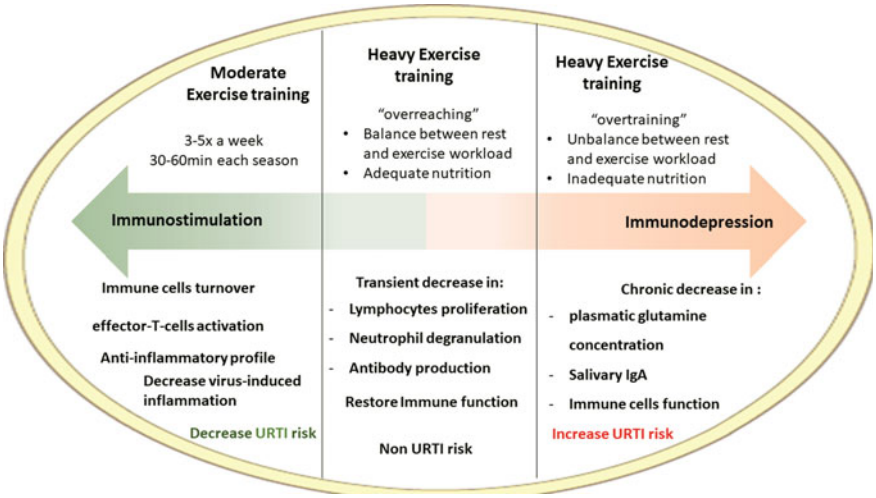


Fig. 2 Immune response according to exercise workload, rest and nutritional status

Western countries. In addition to genetic factors and population aging, lifestyle plays a major role in the development and progression of the "'metabolic syndrome" that represents a cluster of metabolic disorders, including abdominal obesity, dyslipidemia, increased blood pressure, and increased fasting glucose levels [60].

Improved accessibility to healthcare systems and general economic developments that support population aging are believed to increase medical costs to levels no longer sustainable in Western societies. Accordingly, lifestyle modification in the prevention and treatment of metabolic disorders represents one of the most promising approaches that is affordable, nontoxic, and highly efficient compared to medications [61].

A major feature of metabolic diseases is obesity. Several clinical and experimental studies have provided evidence for an association between visceral adipose tissue mass and a condition of chronic systemic and local inflammation. It is suggested that inflammatory processes represent an important cause of many obesity-associated risk factors and metabolic diseases.

There are differences in the risk of obesity based on the heterogeneity of adipose tissue within and among individuals. High visceral and ectopic fat accumulation is shown to be associated with higher cardiovascular risk compared with subcutaneous fat. Due to this distribution of body fat, there is a small subset of apparently metabolic healthy obese individuals [3]. These individuals have preserved insulin sensitivity, low fat storage in the liver and skeletal muscle, normal adipose tissue function characterized by lower adipose tissue infiltration of leukocytes and a physiological adipokine secretion pattern. However, in most cases, excessive body fat accumulation leads to adverse metabolic effects, like adipose tissue inflammation, insulin resistance, impaired glucose tolerance, dyslipidemia, and hypertension [62].

The reason for the pathological changes in the visceral and ectopic adipose tissue is massive tissue expansion resulting from chronic metabolic overload in adipocytes.

Adipocytes represent a type of metabolic cells that not only minimally increase in number, but also increase in size. Subsequently, the chronic overload induces cellular stress pathways and inflammatory signals, including the activation of IκB kinase (IKK), c-jun N-terminal kinase (JNK), and protein kinase R (PKR). Downstream, the phosphorylation of IκB results in the dissociation of IκBα from NF-κB that is translocated into the nucleus inducing the expression of various inflammatory genes [63].

This signaling cascade is suggested to translate a primarily metabolic complication to immunological activation [64]. Simultaneously, the chronically enhanced calorie intake exceeds adipose tissue lipid storage capacity. The fatty acid metabolism products and advanced glycated end-products, accumulating due to hyperglycemia, activate various pattern-recognition such as the Toll-like receptors (TLRs) receptors and the NLRP3 inflammasome in hepatocytes and other tissues, thereby inducing endoplasmatic reticulum stress and aggravating cytokine production. Self-energizing inflammatory processes are initiated accompanied by increased expression and release of cytokines, such as TNF-α, IL-1β, IL-18, and several chemokines that induce the recruitment of leukocytes.

Accordingly, metabolic stress induces an inflammatory switch in the metabolic cells, such as adipocytes and hepatocytes, followed by invasion of leukocytes [63, 65]. The temporal and functional dynamics of adipose tissue residents or invading leukocytes has been partly defined. The increased concentration of pro-inflammatory cytokines and chemokines induces chemotactic recruitment of circulating monocytes that later culminates in the M1 polarization of macrophages that infiltrate the adipose tissue. The condition of chronic hyperlipidemia is also driven by excess fatty acids that bind to fatty acid-binding protein 4 (FABP4) on adipocytes or stromal macrophages in the adipose tissue microenvironment, followed by the induction of other chemokines that primarily recruit M1 macrophages [66, 67].

These cells seem to represent the most important producer of pro-inflammatory cytokines in the adipose tissue during obesity. M1 macrophages that express the surface molecule CD11c are able proliferate during obesity in a monocyte chemotactic protein 1 (MCP-1)-dependent mechanism. CD11c+ macrophages seem to play a major role in the development of insulin resistance and contribute to the formation of crown-like structures that represent a cluster of dead adipocytes surrounded by macrophages [68, 69].

In parallel, other immune cells, such as NK cells and lymphocytes, increase in the adipose tissue. NK cells are important producers of interferon-gamma (IFN-γ) that supports macrophage polarization and pro-inflammatory cytokine production [68, 70]. Regarding T cells, mainly cytotoxic CD8+ cells infiltrate the expanded adipose tissue and contribute to the recruitment, differentiation, and activation of macrophages. In contrast, the levels of regulatory T cells decrease, potentially favoring inflammatory and metabolic complications [71].

Hence, inflammation in the adipose tissue is affected by the imbalance of pro- and anti-inflammatory immune cell homeostasis toward a more pro-inflammatory status. The long-time metabolic surplus during the development of obesity leads to immunometabolic alterations in various other organs and tissues, such as the liver,

brain, skeletal and cardiac muscle, blood vessels, lung, kidney, and gut. However, the adipose tissue is suggested to be an important source of inflammation that at least partially contributes to the induction of systemic inflammatory processes via a "spill over" into circulation [72].

Here, chronically increased levels of pro-inflammatory cytokines, such as TNF-α and IL-6, are involved in the development of other metabolic complications, such as progressive insulin resistance in the skeletal muscle [73]. In particular, TNF-α reduces the level of the inhibitor of IκB, followed by the upregulation of NF-κB and JNK pathways in the muscle. Subsequently, insulin resistance increased through the inhibition of insulin receptor substrate 1 (IRS-1). Non-esterified fatty acids (NEFA) have also been shown to cause insulin resistance in the muscle tissue through increased levels of intramuscular diacylglycerol (DAG) and ceramides.

Here, insulin resistance is also promoted via the upregulation of NF-κB. Moreover, NEFAs induce catabolic responses in the muscle via atrophy-related signaling and protein degradation through impaired activation of the PI3K-Akt pathway [74, 75].

Lifestyle modification appears to be the most appealing approach, and physical activity and exercise represent fundamental components. In general, weight loss is necessary for reducing the cardiovascular risk in obese subjects; however, physical activity is also effective in the treatment of many diseases, irrespective of weight loss. Exercise is well proven to reduce the risk of coronary heart disease in obese subjects. Exercise burns calories and may contribute to a calorie deficit in combination with specific dietary restrictions. Therefore, physical activity plays a pivotal role in long-term weight loss or maintenance to prevent obesity or as an important therapeutic option [76].

In parallel, most exercise regimes significantly improve the functional status and physical fitness. Regular exercise training induces a graded dose response in fitness in both sedentary and obese subjects that is associated with a lower risk for all-cause and cardiovascular disease mortality. It is well known that both moderate endurance as well as resistance exercise training reduces blood pressure in hypertensive individuals. The initiation of regular activity programs in previously inactive subjects also influences dyslipidemia by reducing small, dense LDL and triglyceride particles. In contrast, LDL particle size and high-density lipoprotein (HDL) cholesterol increase. Furthermore, regular physical activity is shown to reduce the ratio of total to HDL cholesterol [77].

Regarding T2D, a considerable association between insulin resistance and low cardiorespiratory fitness has been demonstrated [78]. Data from the *Nurses' Health Study* suggests that the activity time spent per week reduces the relative risk of developing T2D. Similarly, exercise represents an effective tool for the therapeutic management of prediabetes and diabetes [79]. Based on the available data, it is recommended that at least 150 min/week of moderate to vigorous aerobic exercise should be performed in combination with one or two sessions of resistance exercise for prevention [80]. Various other studies, such as the Look AHEAD (Action for Health in Diabetes), has shown various beneficial effects of regular physical activity

in subjects with T2D. Thereby, long-term activity programs in a diabetic population have shown to improve all the aspects of metabolic syndrome, including BMI and HbA1c [81].

Epidemiological studies demonstrate a negative association between the level of physical inactivity and systemic inflammatory status in patients with metabolic diseases. Similarly, physical fitness is negatively associated with the level of inflammatory biomarkers [82]. Accordingly, physical activity, particularly endurance training or combined endurance and strength training programs, seem to interact with the immune system by exerting anti-inflammatory effects. Patients who suffer from metabolic diseases can perform exercise training to lower both systemic and local levels of inflammation that have consistently shown to lower disease activity [83]. Some exercise-effects might address inflammatory processes indirectly, such as by regulating body composition. Maintaining low visceral fat mass prevents the development of adipose tissue dysfunction and inflammation. Regulation of dyslipidemia may improve T-cell receptor signaling and the translocation of MHC molecules for antigen presentation. Furthermore, regular exercise is shown to increase the body's antioxidant defense system that prevents oxidative DNA damage to the immune cells and tissues [84].

In addition to these indirect effects, there are other direct mechanisms for exercise-immune crosstalk. During muscle contraction, various myokines are released into the circulation that exert immune-regulating effects in the circulation or organs and tissues. In response to muscular glycogen depletion, IL-6 is released from the skeletal muscle; this induces the increase of anti-inflammatory cytokines, such as IL-10, IL-1 receptor antagonist (IL-1ra), soluble TNF-α receptors, and inhibits the endotoxin-induced TNF-α production [85].

Similarly, each acute bout of exercise releases stress hormones with anti-inflammatory properties, such as cortisol and adrenaline, by activating both the SNS as well as the HPA axes [86]. While cortisol suppresses the activity of different leukocyte subpopulations, adrenaline attenuates the production of IL-1β and TNF-α. Regarding circulating leukocytes, regular exercise increases the percentage of Tregs in the blood and downregulates TLRs on monocytes and macrophages [87, 88]. Similarly, regular exercise increases the percentage of classical monocytes expressing $CD14^{hi}CD16^{-}$, while an inactive lifestyle promotes an increased percentage of non-classical monocytes characterized by the surface profile $CD14^{low}CD16^{+}$ or $CD14^{hi}CD16^{+}$ [89].

An important mechanism of the immune-regulating potential of exercise is energy expenditure that reduces metabolic stress. In particular, regular exercise training increases adipocyte-specific gene and protein expression of AMPK and PGC-1α followed by an increased β-oxidation and mitochondrial biogenesis [90]. Enhanced lipid turnover decreases metabolic overload and adipocyte dysfunction, thus limiting stress signaling and pro-inflammatory cytokine production in the adipose tissue. Similarly, exercise reduces signals for ER stress-induced inflammation. In parallel, the expression of CD163, a marker for M2 macrophages, increases, suggesting a switch from M1 to M2 macrophage polarization. In line with these observations,

reduced expression of TNF-α and IL-6 in the adipose tissue of diabetic subjects was shown after exercise training [91].

Finally, exercise affects the type of adipose tissue that is generally composed of the following two types of adipose tissues: white adipose tissue (WAT) and brown adipose tissue (BAT). WAT plays a role storing energy and releasing hormones and cytokines that affect metabolism and insulin resistance. BAT, however, expends energy to produce heat through non-shivering thermogenesis, via mitochondrial uncoupling protein 1 (UCP-1) (Bargut et al. 2016). It is noteworthy that exercise can induce a browning process of WAT, leading to increased energy expenditure, thus aiding in the treatment of obesity. Mechanistically, the release of the myokine irisin and activity of the sympathetic nervous system seem to be involved in exercise-induced browning of the adipose tissue. However, the detailed contribution of BAT in the prevention and treatment of metabolic diseases remains to be clarified [92].

Moreover, exercise is an excellent tool for preventing and treating other metabolic and inflammatory diseases, such as neurodegenerative diseases, cardiovascular diseases, and cancer.

4 Exercise for the Prevention and Treatment of Cancer

Cancer is a multifactorial disease driven by chronic inflammation. Aerobic exercise training is considered a very effective tool for inducing an anti-inflammatory response. Thus, nowadays is well established that athletes of long-distance competitions showed reduced risk to many types of cancers. Moore et al. [93] showed that the duration of physical exercise is negatively correlated with the occurrence of 13 types of tumor. Exercise is able to reduce the most common tumors in Western countries, such as cancers of the breast, prostate, colon, pancreas, kidneys, and lungs [94]. It is noteworthy that exercise is less effective in murine models with deletion of p53 [95]. Thus, tumors that have a great genetic trigger and therefore, higher expressions of oncogenes, are less susceptible to exercise-induced prevention [96].

The mechanisms for reducing the risk of cancer are associated with the anti-inflammatory myokines released after exercise with a systemic effect. This anti-inflammatory milieu caused by exercise practice is enough to inhibit tumor generation and growth [97]. Hanahan and Weiberg [98] proposed that inflammation is a hallmark of cancer because the pro-inflammatory signaling can promote resistance to apoptosis and increase tumor cell proliferation.

Other mechanism related to cancer prevention in well-trained subjects is the reduction in the hormones and growth factors, such as sex hormones, insulin, and IGF-1, because exercise training increases the receptors in the target cells and improves the signaling cascade, such as that for insulin. Thus, the circulating levels of these factors can be reduced without affecting the cellular response [94] (Xi et al. 2007).

Most recently, the positive effects of exercise in the treatment of cancer have been shown. Thus, aerobic and resistance exercise can exert effects by reducing the growth of the tumor and metastasis, thus improving the life quality and expectancy [97].

The molecular mechanisms through which exercise induces this anti-tumor response are not completely clear; however, several hypotheses have been proposed. A large number of blood vessels are present in the tumor; however, it is nonfunctional, with reduction in blood flux and generation of hypoxia. Hypoxia is a characteristic of tumor cells, and these reductions in blood flux impair the delivery of immune cells and chemotherapy drugs. Aerobic exercise is able to increase VEGF and improve intra-tumoral perfusion in murine models of breast and prostate cancer [99, 100].

The cell metabolism in tumor is characterized by increased aerobic glycolysis owing to a higher proliferation ratio. Exercise can decrease the availability of energy substrate, particularly special glucose and glutamine, which reduces the supply of nutrients to the tumor. The tumors that have a high metabolic demand are most affected by exercise. In this sense, the metabolic program of tumor cells showed increased in AKT-mTOR axis [101]. Endurance exercise reduces mTOR activation in different types of solid tumors in murine models by reducing the growth factors. Moreover, exercise induces AMPK activation in tumor cells; this activation inhibits the mTOR pathway and inhibits the proliferative pathway. The role of AMPK and the activation of its enzyme is not fully understood. In many types of tumors, AMPK activation decreases tumor growth; however, others studies have shown that this enzyme effectively protects the tumor from oxidative stress and apoptosis [102, 103]. More studies are necessary to clarify the role of AMPK in cancer cells.

Catecholamine released in response to exercise and myokines are probably important for reducing tumor growth and activating the immune system. Pedersen et al. (2016) showed that in a murine model, exercise increased IL-6 and adrenaline that together mobilized NK cells, increasing its activity. It is noteworthy because the levels of IL-6 in intra-tumoral environment are associated with poor prognosis and high malignance. The results not published of our group showed that the aerobic training induces an increase in the IL-6 protein expression in the skeletal muscle and a reduction in the tumor.

The potential therapeutic role of exercise in the reduction of tumor growth (range 45%–67%) in a murine model is well documented. The incubation of several tumor cell lineages with the serum of trained animals reduces in 10%–15% tumor cells proliferation. Furthermore, well-trained athletes have a 40% lower risk of cancer mortality than the general population [94].

The strong evidence of exercise on treatment of cancer patients lead to the Exercise and Sport Science Australian published the position statement in 2019, with the guidelines about the practice of exercise in cancer patients. Several aspects must be considered, such as the type of cancer, pre-status of physical fitness, chemotherapy and radiotherapy, aging, and other co-morbidities; however, all intensities of exercise are preconized during the training. In this statement, the authors suggested at least 20 min for each session (Hayes et al. 2019).

Finally, exercise training is very important in the counter-regulation of the side effects of chemotherapy. This class of drugs causes several undesirable effects in different organs. Doxorubicin, an effective anti-tumor drug widely used in clinical practice, causes substantial toxicity in the heart, kidneys, adipose tissues, liver, and skeletal muscle. In skeletal muscle, patients suffer with proteolysis and fatigue,

mimicking sarcopenia induced by cachexia. Resistance training is an efficient method of reducing weight and skeletal muscle mass loss. Moreover, endurance training mitigates the effects of doxorubicin in the reduction of running capability in mice and protects the decline in oxidative metabolism induced by this drug in the skeletal muscle. Moreover, aerobic training improves the delivery of drugs to the tumor. Some studies have shown that aerobic training is safe and potentiate the chemotherapy treatment reducing the dosage without losing efficacy.

Thus, exercise leads to metabolic reprograming in many tissues of the body and can prevent cancer initialization, thus reducing the cancer risk. Starting a fitness program after cancer diagnosis is safe and recommended. Several studies have shown that exercise reduces cancer growth and metastasis. Finally, exercise is extremely effective for reducing the side effects promoted by anti-tumoral drugs.

5 Conclusion

We discussed the metabolic pathways that induce cellular reprograming, especially with respect to the relationship between the AMPK and mTOR in the skeletal muscle, adipose tissue, and immune cells.

This is an important way to understand how exercise induces mobilization, recruitment, polarization, and production of inflammatory mediators in immune cells. Thus, we showed the state of art in the cellular adaptations induced by acute and training exercise. However, many steps of reprograming are unclear and need be investigated. In sum, exercise is an excellent alternative method for prevention and non-pharmacological treatment of inflammatory chronic diseases (obesity and cancer) that have reached epidemic proportions worldwide.

References

1. Neto JCR, Lira FS, De Mello MT, Santos RVT. Importance of exercise immunology in health promotion. Amino Acids 41(5):1165–1172
2. Pedersen BK (2009) The diseasome of physical inactivity–and the role of myokines in muscle–fat cross talk. J Physiol 587(Pt 23):5559–5568
3. Blüher S, Käpplinger J, Herget S, Reichardt S, Böttcher Y, Grimm A, Kratzsch J, Petroff D (2017) Cardiometabolic risk markers, adipocyte fatty acid binding protein (aFABP) and the impact of high-intensity interval training (HIIT) in obese adolescents. Metabolism 68:77–87
4. Caldeira RS, Panissa VLG, Inoue DS, Campos EZ, Monteiro PA, Giglio BM, Pimentel GD, Hofmann P, Lira FS (2018) Impact to short-term high intensity intermittent training on different storages of body fat, leptin and soluble leptin receptor levels in physically active non-obese men: a pilot investigation. Clin Nutr ESPEN. 28:186–192
5. Inoue DS, Panissa VL, Antunes BM, Oliveira FP, Malta RB, Caldeira RS, Campos EZ, Pimentel GD, Franchini E, Lira FS (2018) Reduced leptin level is independent of fat mass changes and hunger scores from high-intensity intermittent plus strength training. J Sports Med Phys Fitness 58(7–8):1045–1051

6. Lira FS, Dos Santos T, Caldeira RS, Inoue DS, Panissa VLG, Cabral-Santos C, Campos EZ, Rodrigues B, Monteiro PA (2017) Short-term high- and moderate-intensity training modifies inflammatory and metabolic factors in response to acute exercise. Front Physiol 31(8):856
7. Michallet AS, Tonini J, Regnier J, Guinot M, Favre-Juvin A, Bricout V, Halimi S, Wuyam B, Flore P (2008) Methodological aspects of crossover and maximum fat-oxidation rate point determination. Diabetes Metab 34(5):514–523
8. Purdom T, Kravitz L, Dokladny K, Mermier C (2018) Understanding the factors that effect maximal fat oxidation. J Int Soc Sports Nutr 12(15):3
9. Frayn KN (2010) Fat as a fuel: emerging understanding of the adipose tissue-skeletal muscle axis. Acta Physiol (Oxf) 199(4):509–518
10. Jeppesen J, Kiens B (2012) Regulation and limitations to fatty acid oxidation during exercise. J Physiol 590(5):1059–1068
11. Schenk S, Horowitz JF (2006) Coimmunoprecipitation of FAT/CD36 and CPT I in skeletal muscle increases proportionally with fat oxidation after endurance exercise training. Am J Physiol Endocrinol Metab 291(2):E254–E260
12. Yoshida Y, Jain SS, McFarlan JT, Snook LA, Chabowski A, Bonen A (2013) Exercise- and training-induced upregulation of skeletal muscle fatty acid oxidation are not solely dependent on mitochondrial machinery and biogenesis. J Physiol 591(18):4415–4426
13. Shaw CS, Clark J, Wagenmakers AJ (2010) The effect of exercise and nutrition on intramuscular fat metabolism and insulin sensitivity. Annu Rev Nutr 21(30):13–34
14. Watt MJ, Heigenhauser GJ, Dyck DJ, Spriet LL (2002) Intramuscular triacylglycerol, glycogen and acetyl group metabolism during 4 h of moderate exercise in man. J Physiol 541(Pt 3):969–978
15. Daemen S, Gemmink A, Brouwers B, Meex RCR, Huntjens PR, Schaart G, Moonen-Kornips E, Jörgensen J, Hoeks J, Schrauwen P, Hesselink MKC (2018) Distinct lipid droplet characteristics and distribution unmask the apparent contradiction of the athlete's paradox. Mol Metab. 17:71–81
16. Gemmink A, Daemen S, Brouwers B, Huntjens PR, Schaart G, Moonen-Kornips E, Jörgensen J, Hoeks J, Schrauwen P, Hesselink MKC (2018) Dissociation of intramyocellular lipid storage and insulin resistance in trained athletes and type 2 diabetes patients; involvement of perilipin 5? J Physiol 596(5):857–868
17. Gemmink A, Daemen S, Kuijpers HJH, Schaart G, Duimel H, López-Iglesias C, van Zandvoort MAMJ, Knoops K, Hesselink MKC (2018) Super-resolution microscopy localizes perilipin 5 at lipid droplet-mitochondria interaction sites and at lipid droplets juxtaposing to perilipin 2. Biochim Biophys Acta Mol Cell Biol Lipids 1863(11):1423–1432
18. Hawley JA, Krook A (2016) Metabolism: one step forward for exercise. Nat Ver Endocrinol. 12(1):7–8
19. Gerosa-Neto J, Panissa VLG, Monteiro PA, Inoue DS, Ribeiro JPJ, Figueiredo C, Zagatto AM, Little JP, Lira FS (2019) High- or moderate-intensity training promotes change in cardiorespiratory fitness, but not visceral fat, in obese men: a randomised trial of equal energy expenditure exercise. Respir Physiol Neurobiol 266:150–155
20. Keating SE, Johnson NA, Mielke GI, Coombes JS (2017) A systematic review and meta-analysis of interval training versus moderate-intensity continuous training on body adiposity. Obes Rev 18(8):943–964
21. Pedersen BK, Steensberg A, Fischer C, Keller C, Keller P, Plomgaard P, Febbraio M, Saltin B (2003) Searching for the exercise factor: is IL-6 a candidate? J Muscle Res Cell Motil 24(2–3):113–119
22. Pedersen BK, Akerström TC, Nielsen AR, Fischer CP (2007) Role of myokines in exercise and metabolism. J Appl Physiol (1985) 103(3):1093–8.
23. Pedersen BK, Fischer CP (2007) Physiological roles of muscle-derived interleukin-6 in response to exercise. Curr Opin Clin Nutr Metab Care 10(3):265–271
24. Pedersen BK, Febbraio MA (2008) Muscle as an endocrine organ: focus on muscle-derived interleukin-6. Physiol Rev 88(4):1379–1406

25. Pedersen BK, Febbraio M (2005) Muscle-derived interleukin-6–a possible link between skeletal muscle, adipose tissue, liver, and brain. Brain Behav Immun 19(5):371–376
26. Cabral-Santos C, Castrillón CI, Miranda RA, Monteiro PA, Inoue DS, Campos EZ, Hofmann P, Lira FS (2016) Inflammatory cytokines and bdnf response to high-intensity intermittent exercise: effect the exercise volume. Front Physiol 7:509
27. Petersen AM, Pedersen BK (2005) The anti-inflammatory effect of exercise. J Appl Physiol (1985) 98(4):1154–62
28. Cabral-Santos C, Gerosa-Neto J, Inoue DS, Panissa VL, Gobbo LA, Zagatto AM, Campos EZ, Lira FS (2015) Similar anti-inflammatory acute responses from moderate-intensity continuous and high-intensity intermittent exercise. J Sports Sci Med 14(4):849–56
29. Camera DM, Smiles WJ, Hawley JA (2016) Exercise-induced skeletal muscle signaling pathways and human athletic performance. Free Radic Biol Med 98:131–143
30. Marcotte GR, West DW, Baar K (2015) The molecular basis for load-induced skeletal muscle hypertrophy. Calcif Tissue Int 96(3):196–210
31. Gerosa-Neto J, Rossi FE, Campos EZ, Antunes BM, Cholewa JM, Lira FS (2016) Impact of short and moderate rest intervals on the acute immunometabolic response to exhaustive strength exercise: part II. J Strength Cond Res 30(6):1570–1576
32. Rossi FE, Gerosa-Neto J, Zanchi NE, Cholewa JM, Lira FS (2016) Impact of short and moderate rest intervals on the acute immunometabolic response to exhaustive strength exercise: part I. J Strength Cond Res 30(6):1563–1569
33. Izquierdo M, Ibañez J, Calbet JA, Navarro-Amezqueta I, González-Izal M, Idoate F, Häkkinen K, Kraemer WJ, Palacios-Sarrasqueta M, Almar M, Gorostiaga EM (2009) Cytokine and hormone responses to resistance training. Eur J Appl Physiol 107(4):397–409
34. Rossi FE, de Freitas MC, Zanchi NE, Lira FS, Cholewa JM (2018) The role of inflammation and immune cells in blood flow restriction training adaptation: a review. Front Physiol 9(9):1376
35. Inoue DS, Panissa VL, Monteiro PA, Gerosa-Neto J, Rossi FE, Antunes BM, Franchini E, Cholewa JM, Gobbo LA, Lira FS (2016) Immunometabolic responses to concurrent training: the effects of exercise order in recreational weightlifters. J Strength Cond Res 30(7):1960–1967
36. Monteiro PA, Campos EZ, de Oliveira FP, Peres FP, Rosa-Neto JC, Pimentel GD, Lira FS (2017) Modulation of inflammatory response arising from high-intensity intermittent and concurrent strength training in physically active males. Cytokine 91:104–109
37. Panissa VLG, Fukuda DH, de Oliveira FP, Parmezzani SS, Campos EZ, Rossi FE, Franchini E, Lira FS (2018) Maximum strength development and volume-load during concurrent high intensity intermittent training plus strength or strength-only training. J Sports Sci Med 17(4):623–632
38. Robertson AJ, Ramesar KC, Potts RC, Gibbs JH, Browning MC, Brown RA, Hayes PC, Beck JS (1981) The effect of strenuous physical exercise on circulating blood lymphocytes and serum cortisol levels. J Clin Lab Immunol 5(1):53–57
39. Steensberg A, Toft AD, Schjerling P, Halkjaer-Kristensen J, Pedersen BK (2001) Plasma interleukin-6 during strenuous exercise: role of epinephrine. Am J Physiol Cell Physiol 281(3):C1001–C1004
40. Suzuki K, Nakaji S, Yamada M, Liu Q, Kurakake S, Okamura N, Kumae T, Umeda T, Sugawara K (2003) Impact of a competitive marathon race on systemic cytokine and neutrophil responses. Med Sci Sports Exerc 35:348–355
41. Walsh NP, Gleeson M, Shephard RJ, Gleeson M, Woods JA, Bishop NC, Fleshner M, Green C, Pedersen BK, Hoffman-Goetz L, Rogers CJ, Northoff H, Abbasi A, Simon P (2011) Position statement. Part one: immune function and exercise. Exerc Immunol Rev 17:6–63
42. Simpson RJ, Bigley AB, Spielmann G, LaVoy EC, Kunz H, Bollard CM (2016) Human cytomegalovirus infection and the immune response to exercise. Exerc Immunol Rev 22:8–27
43. Nieman DC (1994) Exercise, infection, and immunity. Int J Sports Med 15(Suppl 3):S131–S141

44. Nieman DC (1994) Exercise, upper respiratory tract infection, and the immune system. Med Sci Sports Exer 6(2):128–39
45. Malm C (2006) Susceptibility to infections in elite athletes: the S-curve. Scand J Med Sci Sports 16(1):4–6
46. Peake JM, Neubauer O, Walsh NP, Simpson RJ (1985) Recovery of the immune system after exercise. J Appl Physiol 122(5):1077–1087
47. Batatinha HAP, Biondo L, Lira FS, Castell LM, Rosa-Neto JC (2018) Nutrients, immune system and exercise: where it will take us? Nutrition. https://doi.org/10.1016/j.nut.2018.09.019
48. Saskia Heijnen, Bernhard Hommel, Armin Kibele,and Lorenza S. Colzato (2015) Neuromodulation of aerobic exercise. A Rev Front Psychol 6:1890
49. Lancaster GI, Khan Q, Drysdale PT, Wallace F, Jeukendrup AE, Drayson MT, Gleeson M (2005) Effect of prolonged exercise and carbohydrate ingestion on type 1 and type 2 T lymphocyte distribution and intracellular cytokine production in humans. J Appl Physiol (1985) 98(2):565–71
50. Bishop NC, Walker GJ, Bowley LA, Evans KF, Molyneux K, Wallace FA, Smith AC (1985) Lymphocyte responses to influenza and tetanus toxoid in vitro following intensive exercise and carbohydrate ingestion on consecutive days. J Appl Physiol 99(1327–1335):2005
51. Witard OC, Turner JE, Jackman SR, Kies AK, Jeukendrup AE, Bosch JA, Tipton KD (2014) High dietary protein restores overreaching induced impairments in leukocyte trafficking and reduces the incidence of upper respiratory tract infection in elite cyclists. Brain Behav Immun 39:211–219
52. Costa RJ, Oliver SJ, Laing SJ, Waiters R, Bilzon JL, Walsh NP (2009) Influence of timing of postexercise carbohydrate-protein ingestion on selected immune indices. Int J Sport Nutr Exerc Metab 19:366–384
53. Kunz HE, Spielmann G, Agha NH, O'Connor DP, Bollard CM, Simpson RJ (2018) A single exercise bout augments adenovirus-specific T-cell mobilization and function. Physiol Behav 1(194):56–65
54. Lansford KA, Shill DD, Dicks AB, Marshburn MP, Southern WM, Jenkins NT (2016) Effect of acute exercise on circulating angiogenic cell and microparticle populations. Exp Physiol 101:155–167. https://doi.org/10.1113/EP085505
55. Robson PJ, Blannin AK, Walsh NP, Castell LM, Gleeson M (1999) Effects of exercise intensity, duration and recovery on in vitro neutrophil function in male athletes. Int J Sports Med 20:128–135
56. Simpson RJ, McFarlin BK, McSporran C, Spielmann G, ó Hartaigh B, Guy K (2009) Toll-like receptor expression on classic and pro-inflammatory blood monocytes after acute exercise in humans. Brain Behav Immun 23:232–239. https://doi.org/10.1016/j.bbi.2008.09.013
57. Batatinha HAP, Diniz TA, de Souza Teixeira AA, Krüger K, Rosa-Neto JC (2019) Regulation of autophagy as a therapy for immunosenescence-driven cancer and neurodegenerative diseases: the role of exercise. J Cell Physiol. https://doi.org/10.1002/jcp.28318
58. Minuzzi LG, Rama L, Chupel MU, Rosado F, Dos Santos JV, Simpson R, Martinho A, Paiva A, Teixeira AM (2018) Effects of lifelong training on senescence and mobilization of T lymphocytes in response to acute exercise. Exerc Immunol Rev 24:72–84
59. Lowder T, Padgett DA, Woods JA (2006) Moderate exercise early after influenza virus infection reduces the Th1 inflammatory response in lungs of mice. Exerc Immunol Rev 12:97–111
60. Bhupathiraju SN, Hu FB (2016) Epidemiology of obesity and diabetes and their cardiovascular complications. Circ Res 118:1723–1735
61. Ortega FB, Lavie CJ, Blair SN (2016) Obesity and cardiovascular disease. Circ Res 118:1752–1770
62. Stefan N, Kantartzis K, Machann J, Schick F, Thamer C, Rittig K, Balletshofer B, Machicao F, Fritsche A, Haring HU (2008) Identification and characterization of metabolically benign obesity in humans. Arch Int Med 168:1609–1616

63. Gregor MF, Hotamisligil GS (2011) Inflammatory mechanisms in obesity. Annu Rev Immunol 29:415–445
64. Hirosumi J, Tuncman G, Chang L, Görgün CZ, Uysal KT, Maeda K, Karin M, Hotamisligil GS (2002) A central role for JNK in obesity and insulin resistance. Nature 420(6913):333–336
65. Krüger K, Mooren FC, Pilat C (2016) The immunomodulatory effects of physical activity. Curr Pharm Des 22(24):3730–3748
66. Amano SU, Cohen JL, Vangala P, Tencerova M, Nicoloro SM, Yawe JC, Shen Y, Czech MP, Aouadi M (2014) Local proliferation of macrophages contributes to obesity-associated adipose tissue inflammation. Cell Metab 19(1):162–171
67. Orr JS, Puglisi MJ, Ellacott KLJ, Lumeng CN, Wasserman DH, Hasty AH (2012) Toll-like receptor 4 deficiency promotes the alternative activation of adipose tissue macrophages. Diabetes 61(11):2718–2727
68. Batatinha HAP, Rosa Neto JC, Krüger K (2019) Inflammatory features of obesity and smoke exposure and the immunologic effects of exercise. Exerc Immunol Rev 25:96–111
69. Wouters K, Gaens K, Bijnen M, Verboven K, Jocken J, Wetzels S, Wijnands E, Hansen D, van Greevenbroek M, Duijvestijn A, Biessen EA, Blaak EE, Stehouwer CD, Schalkwijk CG (2017) Circulating classical monocytes are associated with CD11c+ macrophages in human visceral adipose tissue. Sci Rep 15(7):42665
70. Nishimura S, Manabe I, Nagasaki M, Eto K, Yamashita H, Ohsugi M, Otsu M, Hara K, Ueki K, Sugiura S, Yoshimura K, Kadowaki T, Nagai R (2009) CD8+ effector T cells contribute to macrophage recruitment and adipose tissue inflammation in obesity. Nat Med 15(8):914–920
71. Chatzigeorgiou A, Karalis KP, Bornstein SR, Chavakis T (2012) Lymphocytes in obesity-related adipose tissue inflammation. Diabetologia 55(10):2583–2592
72. Krüger K (2017) Inflammation during obesity—pathophysiological concepts and effects of physical activity. Dtsch Z Sportmed 68:163–169
73. Perry BD, Caldow MK, Brennan-Speranza TC, Sbaraglia M, Jerums G, Garnham A, Wong C, Levinger P, Asrar Ul Haq M, Hare DL, Price SR, Levinger I (2016) Muscle atrophy in patients with Type 2 Diabetes Mellitus: roles of inflammatory pathways, physical activity and exercise. Exerc Immunol Rev 22:94–109
74. Aguirre V, Uchida T, Yenush L, Davis R, White MF (2000) The c-Jun NH2-terminal kinase promotes insulin resistance during association with insulin receptor substrate-1 and phosphorylation of Ser307. J Biol Chem 275:9047–9054
75. Liang H, Tantiwong P, Sriwijitkamol A, Shanmugasundaram K, Mohan S, Espinoza S, Defronzo RA, Dube JJ, Musi N (2013) Effect of a sustained reduction in plasma free fatty acid concentration on insulin signalling and inflammation in skeletal muscle from human subjects. J Physiol 591:2897–2909
76. Villareal DT, Chode S, Parimi N, Sinacore DR, Hilton T, Armamento-Villareal R, Napoli N, Qualls C, Shah K (2011) Weight loss, exercise, or both and physical function in obese older adults. N Engl J Med 364(13):1218–1229
77. Kelly AS, Wetzsteon RJ, Kaiser DR, Steinberger J, Bank AJ, Dengel DR (2004) Inflammation, insulin, and endothelial function in overweight children and adolescents: the role of exercise. J Pediatr 145(6):731–6. https://doi.org/10.1016/j.jpeds.2004.08.004. PMID: 15580192
78. Tuomilehto J, Lindström J, Eriksson JG, Valle TT, Hämäläinen H, Ilanne-Parikka P, Keinänen-Kiukaanniemi S, Laakso M, Louheranta A, Rastas M, Salminen V, Uusitupa M (2001) Finnish diabetes prevention study group. Prevention of type 2 diabetes mellitus by changes in lifestyle among subjects with impaired glucose tolerance. N Engl J Med 344(18):1343–50
79. Chae JS, Kang R, Kwak JH, Paik JK, Kim OY, Kim M, Park JW, Jeon JY, Lee JH (2012) Supervised exercise program, BMI, and risk of type 2 diabetes in subjects with normal or impaired fasting glucose. Diabetes Care 35(8):1680–1685
80. Hallal PC, Bauman AE, Heath GW, Kohl HW 3rd, Lee IM, Pratt M (2012) Physical activity: more of the same is not enough. Lancet 380(9838):190–91
81. Di Loreto C, Fanelli C, Lucidi P, Murdolo G, De Cicco A, Parlanti N, Santeusanio F, Brunetti P, De Feo P (2003) Validation of a counseling strategy to promote the adoption and the maintenance of physical activity by type 2 diabetic subjects. Diabetes Care 26(2):404–8

82. Parsons TJ, Sartini C, Welsh P, Sattar N, Ash S, Lennon LT, Wannamethee SG, Lee IM, Whincup PH, Jefferis BJ (2017) Physical activity, sedentary behavior, and inflammatory and hemostatic markers in men. Med Sci Sports Exerc 49(3):459–465
83. Krüger K, Pilat C, Schild M, Lindner N, Frech T, Muders K, Mooren FC (2015) Progenitor cell mobilization after exercise is related to systemic levels of G-CSF and muscle damage. Scand J Med Sci Sports 25:e283–e291. https://doi.org/10.1111/sms.12320
84. Kruk J, Aboul-Enein HY, Kładna A, Bowser JE (2019) Oxidative stress in biological systems and its relation with pathophysiological functions: the effect of physical activity on cellular redox homeostasis. Free Radic Res 53(5):497–521
85. Steensberg A, Fischer CP, Keller C, Moller K, Pedersen BK (2003) IL-6 enhances plasma IL-1ra, IL-10, and cortisol in humans. Am J Physiol Endocrinol Metab 285(2):E433–E437
86. Gleeson M, Bishop NC, Stensel DJ, Lindley MR, Mastana SS, Nimmo MA (2011) The anti-inflammatory effects of exercise: mechanisms and implications for the prevention and treatment of disease. Nat Rev Immunol 11(9):607–615
87. Rada I, Deldicque L, Francaux M, Zbinden-Foncea H (2018) Toll like receptor expression induced by exercise in obesity and metabolic syndrome: a systematic review. Exerc Immunol Rev 24:60–71
88. Weinhold M, Shimabukuro-Vornhagen A, Franke A, Theurich S, Wahl P, Hallek M, Schmidt A, Schinköthe T, Mester J, von Bergwelt-Baildon M, Bloch W (2016) Physical exercise modulates the homeostasis of human regulatory T cells. J Allergy Clin Immunol 137(5):1607–1610
89. Aw NH, Canetti E, Suzuki K, Goh J (2018) Monocyte subsets in atherosclerosis and modification with exercise in humans. Antioxidants (Basel) 19;7(12)
90. DeFronzo RA, Gunnarsson R, Björkman O, Olsson M, Wahren J (1985) Effects of insulin on peripheral and splanchnic glucose metabolism in noninsulin-dependent (type II) diabetes mellitus. J Clin Invest 76:149–155
91. Kawanishi N, Yano H, Yokogawa Y, Suzuki K (2010) Exercise training inhibits inflammation in adipose tissue via both suppression of macrophage infiltration and acceleration of phenotypic switching from M1 to M2 macrophages in high-fat-diet-induced obese mice. Exerc Immunol Rev 16:105–118
92. Rodríguez A, Becerril S, Ezquerro S, Méndez-Giménez L, Frühbeck G (2017) Crosstalk between adipokines and myokines in fat browning. Acta Physiol (Oxf) 219(2):362–381. https://doi.org/10.1111/apha.12686. Epub 2016 Apr 20. PMID: 27040995
93. Moore SC, Lee IM, Weiderpass E, Campbell PT, Sampson JN, Kitahara CM, Keadle SK, Arem H, Berrington de Gonzalez A, Hartge P, Adami HO, Blair CK, Borch KB, Boyd E, Check DP, Fournier A, Freedman ND, Gunter M, Johannson M, Khaw KT, Linet MS, Orsini N, Park Y, Riboli E, Robien K, Schairer C, Sesso H, Spriggs M, Van Dusen R, Wolk A, Matthews CE, Patel AV (2016) Association of leisure-time physical activity with risk of 26 types of cancer in 1.44 million adults. JAMA Intern Me 176(6):816–25
94. Ruiz-Casado A, Martín-Ruiz A, Pérez LM, Provencio M, Fiuza-Luces C, Lucia A (2017) Exercise and the hallmarks of cancer. Trends Cancer 3(6):423–441
95. Colbert LH, Westerlind KC, Perkins SN, Haines DC, Berrigan D, Donehower LA, Fuchs-Young R, Hursting SD (2009) Exercise effects on tumorigenesis in a p53-deficient mouse model of breast cancer. Med Sci Sports Exerc 41(8):1597–1605
96. Jones LW, Kwan ML, Weltzien E, Chandarlapaty S, Sternfeld B, Sweeney C, Bernard PS, Castillo A, Habel LA, Kroenke CH, Langholz BM, Queensberry CP Jr, Dang C, Weigelt B, Kushi LH, Caan BJ (2016) Exercise and prognosis on the basis of clinicopathologic and molecular features in early-stage breast cancer: the LACE and pathways studies. Cancer Res 76(18):5415–5422. https://doi.org/10.1158/0008-5472.CAN-15-3307
97. Hojman P, Gehl J, Christensen JF, Pedersen BK (2018) Molecular mechanisms linking exercise to cancer prevention and treatment. Cell Metab 27(1):10–21. https://doi.org/10.1016/j.cmet.2017.09.015
98. Hanahan D, Weinberg RA (2011) Hallmarks of cancer: the next generation. Cell 144(5):646–674. https://doi.org/10.1016/j.cell.2011.02.013

99. Jones LW et al (1985) (2010) Effect of aerobic exercise on tumor physiology in ananimal model of human breast cancer. J Appl Physiol 108(343–348):48
100. Jones LW et al (1985) (2012) Exercise modulation of the host–tumor interaction in an orthotopic model of murine prostate cancer. J Appl Physiol 113(263–272):49
101. Zhu Z et al (2008) Effectofnonmotorizedwheelrunningon mammary carcinogenesis:circulatingbiomarkers, cellularpro- cesses, andmolecularmechanismsinrats. Cancer Epidemiol Biomarkers Prev 17:1920–1929
102. Kishton RJ, Barnes CE, Nichols AG, Cohen S, Gerriets VA, Siska PJ, Macintyre AN, Goraksha-Hicks P, de Cubas AA, Liu T, Warmoes MO, Abel ED, Yeoh AE, Gershon TR, Rathmell WK, Richards KL, Locasale JW, Rathmell JC1 (2016) AMPK is essential to balance glycolysis and mitochondrial metabolism to control T-ALL cell stress and survival. Cell Metab 23(4):649–62. https://doi.org/10.1016/j.cmet.2016.03.008
103. Vara-Ciruelos D, Russell FM, Hardie DG (2019) The strange case of AMPK and cancer: Dr Jekyll or Mr Hyde? (†). Open Biol 9(7):190099

Immunometabolism and Organ Transplantation

Omar Domínguez-Amorocho, Jean De Lima, and Niels Olsen Saraiva Camara

1 Introduction

Inflammation fundamentally influences short and long-term performance of solid organ allografts. Thus, it is relevant to control inflammatory processes to maintain graft function and survival. Graft inflammation is initiated after ischemia and reperfusion injury and, later, during antigen presentation, where alloantigens are recognized by the recipient immune system, resulting in rejection [1]. Local and systemic production of cytokines and chemokines affect the graft after transplantation and amplify the alloresponse [2]. If the inflammatory response is not resolved, it become chronic, stimulating tissue remodeling with the result of organ fibrosis and a final loss of function and graft failure [1].

Immune cells display a range of metabolic adaptations for energetic requirements and to support growth, expansion and effector functions to maintain the homeostasis or to respond to alarmins, as in the case of inflammation. The role of metabolism in tissue homeostasis has been described in terms of tissue repair and regeneration [3, 4] and in the context of the inflammatory processes involved in organ transplantation [5–7]. Immunometabolism has been described as a pivotal mechanism, for the adaptive and innate immune regulation [8]. There are evidence that metabolic pathways are linked to cell processes like signaling and differentiation leading to different immune

O. Domínguez-Amorocho
Immunometabolism and Inflammation Laboratory, Centro de Biología Molecular Severo Ochoa, Cellular Communication & Inflammation Unit, Madrid, Spain

Department of Molecular Biology, Universidad Autónoma de Madrid (UAM), Madrid, Spain

Institute of Biomedical Sciences, University of São Paulo, São Paulo, Brazil

J. De Lima · N. O. S. Camara (✉)
Laboratory of Transplantation Immunobiology, Department of Immunology, Institute of Biomedical Sciences, University of São Paulo, São Paulo, Brazil
e-mail: niels@icb.usp.br

N. O. S. Camara et al. (eds.), *Essential Aspects of Immunometabolism in Health and Disease*, https://doi.org/10.1007/978-3-030-86684-6_13

cell populations to acquire specific programs according to their state and to micro-environmental signals that will utmost drive cell fate [9].

This chapter outlines some of the metabolic features of immune cells in the context of the allogenic recognition and the immune response in transplantation. Changes in cell metabolism contributing the functional immune responses described in organ transplantation will be also briefly discussed.

2 Metabolic Pathways in the Context of the Immune Response in Transplantation

In homeostatic conditions, cells can use different metabolic pathways, when facing growth factors and nutrient availabilities, and participate in reducing and oxidizing reactions [10]. These changes in metabolic pathways and in generating metabolites have an important effect in the function of immune cells. The activity and importance of such metabolic pathways vary greatly depending on the type and the active state of the cell, affecting several aspects such as development, differentiation, death and cell signaling [11, 12], all of them relevant in the context of transplantation.

In general, six metabolic pathways have been studied in more details. The glycolytic pathway or glycolysis is involved in the glucose transport from extracellular compartment to generate pyruvate and other metabolites as a result of a series of enzymatic reactions. Pyruvate is imported into the mitochondria and consequently converted to acetyl-CoA to enter into the tricarboxylic acid cycle (TCA) to produce NADH and FADH2 for ATP generation. Also, pyruvate can be metabolized by lactate dehydrogenase (LDH) into lactate, regenerating the NAD+ consumed during the generation of pyruvate from glucose [13]. The pentose phosphate pathway (PPP) generates precursors for nucleotide and amino acid biosynthesis and to control oxidative stress. The PPP reactions divide into an oxidative and non-oxidative branch, the oxidative branch converts glucose 6-phosphate into ribulose 5-phosphate, carbon dioxide, and NADPH. For instance, the non-oxidative branch metabolizes the glycolytic intermediates to ribose 5-phosphate for nucleic acids and amino acids synthesis [14]. In the fatty acid oxidation pathway, β-oxidation of fatty acid yields large amounts of acetyl-CoA, NADH and FADH2 to be used in the tricarboxylic acid (TCA) cycle and the electron transport chain to generate ATP [15]. In contrast, the fatty acid synthesis (FAS) pathway is used to generate lipids for cellular growth, membrane production and other important lipid-based components crucial for proliferation [16]. Inside the mitochondria, acetyl-CoA could be used as a precursor for citrate, which can be then exported to the cytosol and converted to malonyl-CoA and finally to palmitate, to form phospholipids, or as a substrate for proteins acylation and cholesterol biosynthesis and used to create or repare cell membranes [13].

Transplantation is a therapeutic alternative that extent the quality of life of individuals with organ failure. In terms of the immune response, allograft recognition

occurs in two steps, the first one mediated by cells and compound of the innate immunity and the second one mediated by the adaptive immune system, mainly T cells, which are necessary and sufficient to induce organ transplant rejection of allografts [17]. During transplantation a cascade of signals drive the migration of dendritic cells (DCs) and lymphocytes into lymph nodes and transplanted tissues for the efficient generation of alloimmune responses [18]. Therefore, alloantigen-dependent immune responses can critically impair its success. Immune cells secrete factors that are important on microenvironment, conditions like nutrient competition, oxygen consumption, microbiota and metabolite production from several tissues have an important effect on the immune cell fate, which leads to pro-inflammatory response by immune cells and alteration into the tissues when this response happen [19, 20].

DCs link innate responses to adaptive immunity, by controlling activation and polarization of effector T helper and regulatory T cell (Treg) responses during the allogeneic recognition. During DCs maturation and activation, their metabolic profile shifts from glycolysis to oxidative phosphorylation (OXPHOS), however, some glycolytic intermediates enter into the PPP, to support protein biosynthesis and NADPH generation, and into the TCA cycle to maintain lipid and macromolecule biosynthesis [6, 21, 22] (Fig. 1.).

T cell-mediated graft alloresponses are critical for graft rejection or acceptance. Early activation steps are defined by an increased glycolytic rate, glutamine, and PPP metabolism, however, protein, lipid and nucleic acids synthesis have been demonstrated as important pathways related with the development of T cells response during graft rejection processes [6, 23].

In vitro studies have shown that glucose and glutamate are important nutrients for effector responses because glucose uptake deficiency impairs effector function and proliferation of CD4+ T cells while Tregs are enriched and functionally unaffected [24]. Glutamate metabolism has also been involved in Th1 and Th17 effector

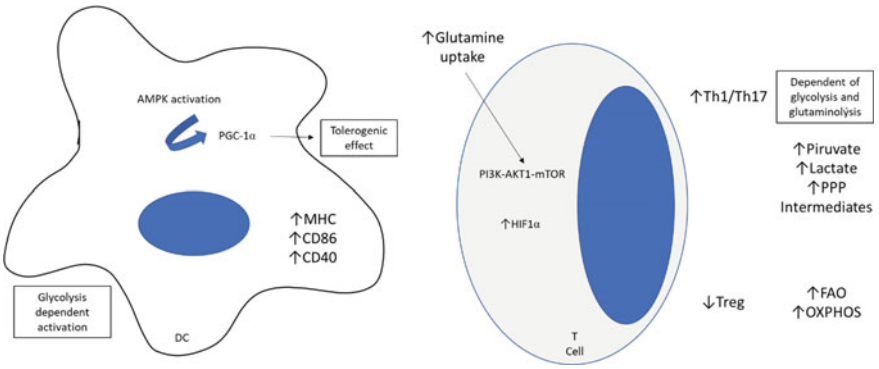


Fig. 1 DCs and T cells metabolic events during immunological synapse. DCs control activation and polarization of effector T helper and regulatory T cell (Treg) responses during allogeneic recognition. Glucose and glutamate are important nutrients for effector responses and they have been involved in Th1, Th17 and Treg differentiation

T cells differentiation, but apparently, it does not seem to be critical for Tregs differentiation [25]. In the context of transplantation, it was demonstrated that glycolysis and glutamine metabolism inhibition promoted allograft survival by suppression of CD4+ and CD8+ effector T cell responses and preservation of immune regulation [6, 26, 27] (Fig. 1).

3 Immunometabolic Events in Transplantation

3.1 Metabolic Changes in Ischemia/Reperfusion

Immune system activation is an early event after organ transplantation and its close related to tissue damage and cell death in transplanted organs. However, tisular cell death is a process that contributes to increase graft inflammation and dead cells as well as the repair of damaged tissue following transplantation [28].

Ischemia/reperfusion injury is an unavoidable consequence after solid organ transplantation which influences short- as well as long-term graft outcome. Energy metabolism, cellular changes of the mitochondria and cellular membranes and cell death are underlying mechanisms involved in this injury [29].

Ischemia has been related with two major underpinning conditions: a complex metabolic shift and a response to oxygen availability. Some studies have demonstrated that ischemic conditions are related to an increasing of glucose transportation and glycolysis [30], excess of extracellular and cytosolic lactate and as, a compensatory mechanism, activation of AMPK signaling to elicit an anti-inflammatory response [31, 32]. Low oxygen rates related to ischemia have been associated with HIF1α activation [33, 34], succinate accumulation and mitochondrial ROS generation [35] (Fig. 2).

Ischemia/reperfusion injury has been linked to mitochondrial damage by increasing of cytoplasmic concentrations of H^+, Na^+ and Ca^{2+}, associated with ATP reduction by ATPases inhibition [36, 37]. In addition, enhanced ROS generation and subsequent peroxidation of phospholipids of the inner mitochondrial membrane disrupt the electron flow, by altering the electron transport chain [38]. ROS-mediated signaling also activates innate immune responses by modulation of MAP kinases, NF-κB, JAK/STAT transducers and nitric oxide (NO) signaling, eventually leading to the amplification of the inflammatory response, fibrosis and loss of the organ function [39].

Increased levels of casein kinase 2α (CK2α) and, consequently, mitophagy inhibition have been also related to ischemia/reperfusion injury, resulting in mitochondrial genome damage, electron transport chain complex (ETC) inhibition, mitochondrial biogenesis alteration and induction of oxidative stress and mitochondrial apoptosis [40].

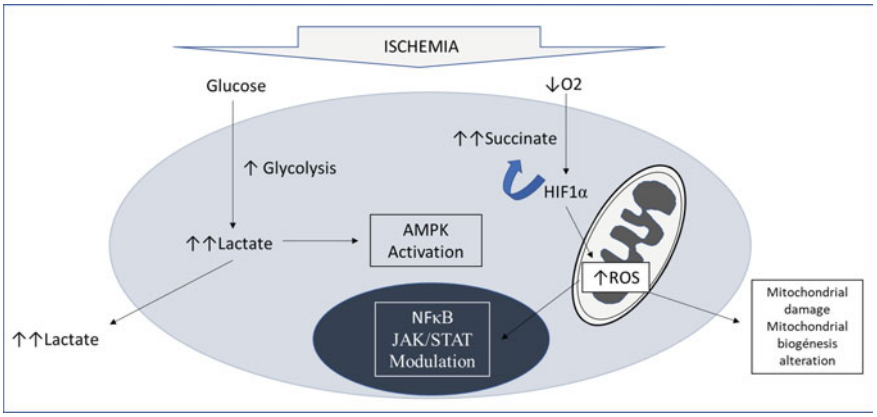


Fig. 2 Metabolic effects of ischemia in cell behaviour during transplantation. During tranaplantation, ischemic conditions related to alterations in glucose transportation, glycolysis and oxigen availability activate nuclear inflammatory mechanisms and generate mitochondrial alterations and mitochondrial damage

3.2 Metabolic Regulation During Acute Rejection

Allogenic T cell responses are considered to be critical for short and long-term outcomes for solid organ transplants [28, 41]. In transplantation, alloantigens are recognized by two main pathways, the indirect (donor-derived antigens are proccesed and presented by receptor´s APCs) and the direct (receptor´s T cells recognize alloantigen/donor MHC on donor APCs) [42].

After activation, DCs undergo a metabolic shift to glycolysis aquiring a pro-inflammatory phenotype, however, memory T cells metabolism displays similarities with Treg cells and M2 macrophages, relying on FAO to meet their metabolic demands [43].

DAMPs recognition during TLR-driven DC activation is a crucial aspect in transplantation. Diverse studies have showed that glycogen metabolism supports the initial effector functions of TLR-activated DCs [44, 45]. In addition, DCs activation is glycolysis dependent, thus, glycose blockade promotes a reduction in the expression of MHC-I and, -II and co-stimulatory molecules like CD86 and CD40, which are fundamental signals during graft antigen presentation steps [45, 46].

Some studies have demonstrated that Th17 cells together with Th1 and Th2 cells play an important role in mediating allograft rejection [47]. During acute rejection, it has been described that frequencies of Th1-Th17, as well as, levels of circulating IFN-γ and IL-17 are increased in contrast with reduced levels of Treg cells [48–50]. During T cell differentiation and activation Th1, Th17 and Tregs display different metabolic patterns. Th1 and Th17 cells have been described more dependent of glycolysis and glutaminolysis, with high levels of pyruvate, lactate and PPP intermediates. Tregs, by their side, rely more on fatty acid oxidation and OXPHOS to supply their energetic demands [51–53].

The effect of maintained inflammation on immune cells is related to changes in cellular metabolism that shift the cells towards aerobic glycolysis, signaling via the phosphoinositide 3-kinase (PI3K–AKT1–mTOR) axis and upregulation of the transcription factors, e.g. MYC and HIF1α. These processes increase amino acid and glutamine uptake to maintain glutaminolysis and glycolysis resulting in T cell activation and inflammatory cytokine production. Indeed, inhibition of glycolysis, either by blocking mTORC1 or downstream HIF1α signaling, promotes a switch from Th1 and Th17 to Treg differentiation [54, 55].

Transplant recipients receive therapeutic doses of immunosuppressant drugs to maintain the integrity of tissues and organs and to avoid rejection. These drugs have important effect on the metabolic behavior of the immune cells. The calcineurin inhibitor therapy is the main immunosuppressive regimens with important effects on cellular enzymatic rate (mediate by cytochrome P450) and regulation of IL-2 production by immune cells [56]. mTOR inhibitors, trough glycolytic metabolic reprograming and preventing cell proliferation by inhibiting of growth factor–mediated T-cell proliferation in response to IL-2 and by blocking the metabolic shift required for the Th17/Treg balance [57–59].

3.3 Metabolic Features in Chronic Rejection and Fibrosis

Chronic allograft injury is identified by vascular changes, interstitial fibrosis and organ structures' atrophy. Fibrosis causes organ damage by a series of cellular and molecular responses to sustained tissue damage. Tissue inflammation often triggers fibrosis by inducing epithelial- and endothelial-to-mesenchymal transitions [60].

Some studies described that IL-33 is released as a response of tissue damage and regalates macrophage differentiation during chronic rejection, preventing disruption of the TCA cycle that shifts macrophage metabolism to anaerobic glycolysis and generates proinflammatory metabolites [61, 62]. Macrophage stimulation by IL-33 increases OXPHOS and FA uptake, supporting reparative and regulatory myeloid cell functions [61].

During chronic rejection, alloantigens are continually processed by recipient APCs, during this process, it has been described that the metabolic profile governing these late chronic responses involves an skewed response from glycolysis and with focus on OXPHOS, what seems to be related to FAO process [55].

3.4 Role of Immune Cell Metabolism in Tolerance Induction

Tolerance, in terms of transplantation, has been defined by different aspects, highlighted between them: 1. Allograft must be accepted without chronic rejection signals, and, 2. Recipient should be able to maintain immune responses against

any other foreign antigen without affectation of the transplanted organ [63]. Transplantation tolerance maintenance is, in fact, the biggest goal in the transplantation field.

Tolerogenic DCs metabolism has been poorly studied in graft transplantation field. However, new topics linking DCs metabolism and anti-inflammatory profile were established in different models and tissues. Recently, arginine and tryptophan pathways were showed to be two important pathways in the development of an anti-inflammatory response. Thus, scientists discovered that arginase 1 (Arg1) expression and activity are important to indoleamine 2,3-dioxygenase 1 (IDO1) phosphorylation, and consequent activation of IDO1 signalling, which promotes tolerogenic and a protection profile in sensitized mice with the HY peptide (containing the H-2D^{b} epitope of male minor histocompatibility antigen complex) [64]. Then, these findings suggested that Arg1 and IDO1 interlinked functions can be extend to transplantation model.

Regarding DCs fate, activation of AMPK signaling by peroxisome proliferator-activated receptor gamma coactivator (PGC) enhance PGC-1α activity. The reduction of HIF-1α activity by PGC-1α increased activity has been observed as an important factor for tolerogenic DCs generation [46, 65].

PPARγ and Wnt/β-catenin, in cDCs of adipose tissue, have important immunoregulatory functions on inflammation. cDC1 and cDC2 control IL-10 and homeostasis of cDC themselves through Wnt/β-catenin and PPARγ, respectively [66]. In addition, Wnt/β-catenin pathway also showed to be extremely important to generate Treg, trough kynurenine secretion out at the microenvironment [67]. Both mechanisms were already seing in transplantation contexts [68], but additonal interactions need to be explored in graft transplantation models.

It has been showed that tolerogenic DCs, generated by RelB (NF-kB subunit) silencing, have the capacity of downregulate Th1 responses, increase T cell apoptosis and generate Treg, which prevent allograft rejection and prolonged survival in a murine model of heart transplantation [69]. In addition, it has been showed that DCs treatment with an analog of vitamin D (1, 25(OH)2D3) promoted a tolerogenic phenotype, characterized by a reduced expression of costimulatory molecules, reduction of IL-12 production and enhanced IL-10 secretion. These tolerogenic DCs promoted graft acceptance in a heart transplantation murine model, associated with a higher frequency of Treg in secundary lymphoid organs [70].

4 Key Metabolic Sensors in Transplantation

Different cell signaling and metabolic pathways participate in the modulation of the allogeneic immune response, some of these main pathways have been described in the context of the inflammatory response and have been studied in application fields like transplantation. Several molecules are involved in every metabolic pathway; however, some factors are key for nutrient sensing and modulation of metabolic programming and reprogramming of immune cells.

mTOR pathway is interlinked to ensure coordinated regulation of cellular metabolic responses, regulating signal intensity and duration of nutrient availability and microenvironmental signaling input [71, 72]. Thus, mTOR pathway can influence proinflammatory cytokine production and Th1/Th17 polarization, by blocking the release of regulatory cytokines like IL-10 and by altering the frequencies of Treg and tolerogenic DCs, factors that promote an accelerated graft rejection [43, 44, 73, 74].

PCG1-α is considered as an important molecule in the context of the metabolic switch during the immune response [75–78]. In the context of transplantation, it has been show in macrophages that PGC1-α expression modulate IL-1α and IL-1β production [79], which are produced as a response by PAMPs recognition in transplantation procedures and cellular rejection response [80]. In addition, it has been described that reductions of PGC1-α levels in CD8+ T foster an exhausted profile, which lead to TCR signal decreased, less pro-inflammatory production and low cytotoxic efficiency, which have an important role at the graft rejection processes [81, 82]. In the same way, CD4+ T cells with low PCG1-α expression have a decreased in proliferation and less cytokines production [15, 79, 83, 84] and could have a pivotal role in the transplantation outcome.

The peroxisome proliferator-activated receptors (PPARs) are members of the nuclear-hormone-receptor superfamily that transduce environmental, nutritional and inflammatory signals events at gene transcription level in T and B cells, macrophages and DCs [85–87], 163]. PPARγ can influence the maturation of DCs, whereas PPARγ is functionally more activated, thereby promoting high levels of CD86. In contrast, it appears that PPARγ activation in DCs also decreased CD80 expression and IL-12 production [40, 85]. It has been showed that PPARγ activation in DCs might influence effector CD4+ T cells activation and polarization through its ability to alter their phenotype and cytokine production [88], which controls the immune response in kidney and heart transplantation models [89].

PPARγ negatively regulates T cells activation and proliferation by decreasing TCR stimulation, mainly through the NFAT signal inhibition and subsequent IL-2 production inhibition [90]. It has been showed that treatment with PPARγ agonists drugs unravel decreased T cell activation in inflammatory environments such as transplantation in mice models, in contrast, use of PPARγ antagonist leads to an increased IFN-γ+CD4+ T cells and to a decreased numbers of CD4+ Foxp3+ T cells [91]. Thus, modulation of Treg/Th1 cells activation axis may help the acceptation of solid organs transplants.

Oxygen availability is one of the factors that are important in terms of cell fate or function, and "oxygen sensors" such as theHIF-1α. This factor has an defined role in many checkpoints in the glycolysis pathway [92]. In DCs, HIF-1α leads to metabolic reprogramming toward glycolysis, increasing the CCR7 expression, essential for migration to draining lymph nodes, as a response for alloantigens during recognition and rejection response [93]. More than ten years ago, it was observed that donors' treatment with prolyl-hydroxylases (PHDs), a HIF-1α and HIF-2α inhibitor, prolonged survival in kidney transplant murine models and studies of physiological responses to hypoxia [94–96].

5 Sirtuins as "Metabolic Sensors and Regulators" in Transplantation

Histone deacetylases (HDACs) are biological molecules responsible for removing acetyl groups from histone´s lysine residues, resulting in chromatin condensation and repression of the transcription [97]. Sirtuins are NAD+ -dependent HDACs, and they are divided into Sirtuin 1–7 (Sirt1–7) [98].

In terms of the study of sirtuins, Sirt1 and Sirt3 have been deeply studied in different cell types, specially in immune cells. Sirt1 and Sirt3 regulate NF-κB, a transcription factor involved in regulation of inflammation and immune cell proliferation [99–102]. By their side, Sirt3 overexpression is related to an increase of the mitochondrial oxidative capacity with a resulting reduction of ROS levels [103], having a concomitant effect on cell activation and function in the context of transplantation [104].

Sirt1 has been identified as an important molecule in cellular metabolism and in immune regulation and their activation has proved protective effects on multiple organs submitted to oxidative stress, including kidneys [105–109], by their side, Sirt1 knockout (KO) mice have been show aggravation in diabetic nephropathy and acute kidney injury [106, 110].

Although some studies have demonstrated that Sirt1 has a role in the suppression of inflammatory responses [111], others have been demonstrated an important proinflammatory role for Sirt1 mainly in the context of Th17 cell differentiation. By deacetylation of RORγt and Foxp3, Sirt1 increases RORγt and reduces Foxp3 activity, promoting Th17 cell development establishing favorable conditions for the development of autoimmunity and graft rejection [112, 113], some of these fondings could suggest that Sirt1 inhibition may represent a potent therapeutic alternative for the treatment of Th17 cell–mediated graft rejection [114].

Some studies have showed a negative regulation of Treg activity by Sirt1 due to deacetylation of three sites on Foxp3 [115]. Strategies to inhibit Sirt1 may enhance Treg activities and provide a therapeutic approach to treat allograft rejection. Sirt1 deletion results in enhanced Foxp3 expression, increased Treg suppressive activity, and longer cardiac allograft survival from fully mismatched major histocompatibility complex (MHC) donors in a murine model. However, functionality of effector T cells was not affected as determined by proliferation, activation, or production of IL-2, IL-4, IL-17, or IFN-gamma [116]. Activated Treg cells downregulate Sirt1, to stabilize Foxp3 expression and Treg phenotypes [117], which may have clinical benefits in transplantation [118–120]. In Human DCs, Sirt1-mediated histone deacetylation of Rel family proteins to the *il12a* promoter and IL-12p70/IL-23 production, which might modulate the Th1/Th17 balance [121].

Sirt1 deletion in T cells improved allograft survival and also lead to the induction of tolerance to a subsequent heart transplantation challenge, maintaining the ability to reject third-party cardiac allografts [122]. Treg role in prevention of allograft rejection provides a rationale for Sirt1 targeting in the context of transplantation [123].

In addition, Sirt3, a mitochondrial sirtuin, promotes the deacetylation of endogenous factors that are important for autophagosome maturation inhibition and induced NLRP3 formation, two relevant components related to organ rejection [80, 124]. Other studies have been showed that T cells isolated from Sirt3 KO mice were found less activated, with reduced CXCR3 expression and ROS production, which led a reduced GVHD severity in different murine models [125].

Taken together, sirtuins, mainly Sirt1 and 3, have been showed important roles in manipulated the immune cells phenotype and function, through the metabolism. However, still exist several gaps to be answered about sirtuins and immunometabolism, highlighting the importance of new studies about these molecules on transplantation models.

6 Trained Immunity in Organ Transplantation

Trained immunity has been defined as the functional reprogramming of innate immune cells leading to an altered response towards a second challenge after the return to a non-activated state. These secondary response can be more or less strong than primary, conferring context- and time-adjusted responses [126].

Cellular metabolism has an important role in trained immunity [127–129]. Indeed, nutrients and metabolites modulate the epigenetic and the metabolic reprogramming increasing glycolytic cell rates mediated by mTOR, AKT and HIF1α [129–131] with an enhanced responsiveness to subsequent stimulation. Also, derived metabolites from the TCA, like α-ketoglutarate or itaconate, promote anti-inflammatory macrophages polarization facilitating tolerance after IFNγ-mediated macrophage activation [126, 132–135].

Some signaling pathways have been described in context of trained immunity. These pathways could be altered or modified by different stimuli or conditions as transplantation, some of these are: (1) Dectin-1/vimentin/HMGB1, (2) infection and NOD2 [17].

Dectin-1 is a C-type lectin innate immune receptor expressed on macrophages, DCs, and epithelial cells, which recognizes β-glucans on common transplant-associated pathogens and recently has been described as a crucial tolerogenic receptor binding several annexins exposed on apoptotic cells [136]. Following a second stimulation, dectin-1 "trained" macrophages upregulate glycolysis and secrete elevated levels of IL-6 and TNFα [137], cytokines that are related to organ transplant rejection [138]. Vimentin, an endogenous protein involved in wound healing, is a dectin-1 ligand [139, 140]. Some studies have shown that vimentin is upregulated and exposed on the surface of apoptotic cells after transplantation and is associated with acute and chronic rejection [141–144]. Necrosis is a result of ischemia/reperfusion injury (IRI) [145], whereas necrotic cells release high levels of mobility group box 1 (HMGB1) protein, other important ligand for dectin-1 [146–148].

Nod-like receptor 2 (NOD2) is an intracellular sensor for bacterial components [149] and viral RNA [150], showing an important link with solid organ transplantation because infection aquisition has been associated with graft rejection [151, 152]. Early studies demonstrated that microbial components or infections induce NOD2-dependent response, which occurs via epigenetic and metabolic reprogramming of innate immune cells. It has been described that trained immune increases histone methylation at the IL-6 and TNFα promoters, and upregulates glycolytic pathways, both associated to an elevated secretion of these pro-inflammatory cytokines [153, 154].

7 Metabolic Conditioning of Microbiome in Transplantation

The "microbiome" embodies a biological ecosystem that encompasses the collective ensemble of bacteria, viruses, fungi, and other microorganisms (ie microbiota) together with their metabolites, degradative byproducts, and genomes [155]. Different studies have showed that gut microbiota has an important role in the immune system having an effect on the development and functionality of gut-associated lymphoid tissues (GALT) [156, 157]. Bacterial metabolites has the capacity to regulate immune responses, some of these molecules produced by gut bacteria are tryptophan metabolites and short chain fatty acids (SCFA) [158].

Tryptophan metabolites activate the aryl hydrocarbon receptor (AhR) present in immune cells. For T-cells, AhR activation is important for Th17 differentiation but it has a suppressor effect on Treg differentiation [159], also, AhR activation promotes monocyte differentiation into DCs over macrophages [160]. In addition, *In-vivo* assays has showed that dietary indoles have a suppressor effect on Th17 differentiation but increase Treg responses [161].

SCFAs (acetate, propionate, and butyrate), are important for Treg homeostasis and also for the control of the inflammatory response by gut macrophages, through the intracellular receptor PPARγ; the surface proteins GPR41 and GPR43 in neutrophils; and the butyrate receptor GPR109a (important in the induction of IL-10 producing T cells) [162, 163]. SCFAs also regulate DCs, thus, human monocyte-derived DCs cultured with C3 or C4 were less inflammatory with decreased production of pro-inflammatory cytokines and chemokines [164, 165].

The role of microbiota in cell activation and differentiation confers it a major importance in organ transplantation because microorganisms create their own microenvironments and provide signals that render DC and T cell interactions. DCs can interact with commensal bacteria and induce tolerogenic macrophages or promote local Treg cells expansion [166].

8 Final Conclusions

- Metabolic conditions have an important effect on differentiation and activation of immune cells. In homeostatic or inflammatory conditions, immune cells use different metabolic pathways to supply energetic demands and to proliferate, in this context nutrient availability and uptake control the immune cell fate.
- During the initial and chronic phases of transplantation, the complex metabolic shift and the response to oxygen availability are related to an increasing of glucose transportation and glycolysis, excess of extracellular and cytosolic lactate and mitochondrial damage, suggesting interesting metabolic targets in the context of the allogenic immune response.
- Metabolic conditioning of tolerogenic DCs and Treg generation has showed a "preventive" resource for allograft rejection and prolonged survival factors in different models of transplantation.
- Epigenetic and metabolic pathways, in terms of the immune conditioning, are both close related. Sirtuins and other epigenetic factors have an important role on cell fate and could be studied deeply in terms of prevent graft rejection.

References

1. Gueler F, Gwinner W, Schwarz A, Haller H (2004) Long-term effects of acute ischemia and reperfusion injury. Kidney Int 66(2):523–527. https://doi.org/10.1111/j.1523-1755.2004.761_11.x
2. Furuichi K, Wada T, Kaneko S, Murphy PM (2008) Roles of chemokines in renal ischemia/reperfusion injury. Front Biosci 13:4021–4028. https://doi.org/10.2741/2990
3. Eming SA, Wynn TA, Martin P (2017) Inflammation and metabolism in tissue repair and regeneration. Science 356(6342):1026–1030. https://doi.org/10.1126/science.aam7928
4. Gaber T, Strehl C, Buttgereit F (2017) Metabolic regulation of inflammation. Nat Rev Rheumatol 13(5):267–279. https://doi.org/10.1038/nrrheum.2017.37
5. Allocco JB, Alegre ML (2020) Exploiting immunometabolism and T cell function for solid organ transplantation. Cell Immunol 351. https://doi.org/10.1016/j.cellimm.2020.104068
6. Domínguez-Amorocho O, Takiishi T, da Cunha FF, Camara NOS (2019) Immunometabolism: a target for the comprehension of immune response toward transplantation. World J Transplant 9(2):27–34. https://doi.org/10.5500/wjt.v9.i2.27
7. Tanimine N, Turka LA, Priyadharshini B (2018) Navigating T-cell immunometabolism in transplantation. Transplantation 102(2):230–239. https://doi.org/10.1097/tp.0000000000001951
8. Mathis D, Shoelson SE (2011) Immunometabolism: an emerging frontier. Nat Rev Immunol 11(2):81. https://doi.org/10.1038/nri2922
9. Makowski L, Chaib M, Rathmell JC (2020) Immunometabolism: from basic mechanisms to translation. Immunol Rev 295(1):5–14. https://doi.org/10.1111/imr.12858
10. Buck MD, Sowell RT, Kaech SM, Pearce EL (2017) Metabolic instruction of immunity. Cell 169(4):570–586. https://doi.org/10.1016/j.cell.2017.04.004
11. Hosomi K, Kunisawa J (2020) Diversity of energy metabolism in immune responses regulated by micro-organisms and dietary nutrition. Int Immunol 32(7):447–454. https://doi.org/10.1093/intimm/dxaa020

12. Kelly PN (2019) Metabolism as a driver of immune response. Science 363(6423):137–139. https://doi.org/10.1126/science.363.6423.137-j
13. Palmer CS, Ostrowski M, Balderson B, Christian N, Crowe SM (2015) Glucose metabolism regulates T cell activation, differentiation, and functions. Front Immunol 6:1. https://doi.org/10.3389/fimmu.2015.00001
14. Pearce EL, Pearce EJ (2013) Metabolic pathways in immune cell activation and quiescence. Immunity 38(4):633–643. https://doi.org/10.1016/j.immuni.2013.04.005
15. O'Neill LA, Kishton RJ, Rathmell J (2016) A guide to immunometabolism for immunologists. Nat Rev Immunol 16(9):553–565. https://doi.org/10.1038/nri.2016.70
16. Stincone A, Prigione A, Cramer T, Wamelink MM, Campbell K, Cheung E, Olin-Sandoval V, Grüning NM, Krüger A, Tauqeer Alam M, Keller MA, Breitenbach M, Brindle KM, Rabinowitz JD, Ralser M (2015) The return of metabolism: biochemistry and physiology of the pentose phosphate pathway. Biol Rev Camb Philos Soc 90(3):927–963. https://doi.org/10.1111/brv.12140
17. Ochando J, Fayad ZA, Madsen JC, Netea MG, Mulder WJM (2020) Trained immunity in organ transplantation. Am J Transplant 20(1):10–18. https://doi.org/10.1111/ajt.15620
18. Johnson AR, Milner JJ, Makowski L (2012) The inflammation highway: metabolism accelerates inflammatory traffic in obesity. Immunol Rev 249(1):218–238. https://doi.org/10.1111/j.1600-065X.2012.01151.x
19. Dasu MR, Jialal I (2011) Free fatty acids in the presence of high glucose amplify monocyte inflammation via Toll-like receptors. Am J Physiol Endocrinol Metab 300(1):E145-154. https://doi.org/10.1152/ajpendo.00490.2010
20. Martinez-Llordella M, Mastoridis S (2018) Immunometabolism: novel monitoring and therapeutic approach in transplantation. Transplantation 102(2):187–188. https://doi.org/10.1097/tp.0000000000001988
21. Sim WJ, Ahl PJ, Connolly JE (2016) Metabolism is central to tolerogenic dendritic cell function. Mediators Inflamm 2016:2636701. https://doi.org/10.1155/2016/2636701
22. Wrenshall L (2003) Role of the microenvironment in immune responses to transplantation. Springer Semin Immunopathol 25(2):199–213. https://doi.org/10.1007/s00281-003-0138-y
23. Qu Q, Zeng F, Liu X, Wang QJ, Deng F (2016) Fatty acid oxidation and carnitine palmitoyltransferase I: emerging therapeutic targets in cancer. Cell Death Dis 7(5). https://doi.org/10.1038/cddis.2016.132
24. Macintyre AN, Gerriets VA, Nichols AG, Michalek RD, Rudolph MC, Deoliveira D, Anderson SM, Abel ED, Chen BJ, Hale LP, Rathmell JC (2014) The glucose transporter Glut1 is selectively essential for CD4 T cell activation and effector function. Cell Metab 20(1):61–72. https://doi.org/10.1016/j.cmet.2014.05.004
25. Nakaya M, Xiao Y, Zhou X, Chang JH, Chang M, Cheng X, Blonska M, Lin X, Sun SC (2014) Inflammatory T cell responses rely on amino acid transporter ASCT2 facilitation of glutamine uptake and mTORC1 kinase activation. Immunity 40(5):692–705. https://doi.org/10.1016/j.immuni.2014.04.007
26. Lee CF, Lo YC, Cheng CH, Furtmüller GJ, Oh B, Andrade-Oliveira V, Thomas AG, Bowman CE, Slusher BS, Wolfgang MJ, Brandacher G, Powell JD (2015) Preventing allograft rejection by targeting immune metabolism. Cell Rep 13(4):760–770. https://doi.org/10.1016/j.celrep.2015.09.036
27. Nguyen HD, Chatterjee S, Haarberg KM, Wu Y, Bastian D, Heinrichs J, Fu J, Daenthanasanmak A, Schutt S, Shrestha S, Liu C, Wang H, Chi H, Mehrotra S, Yu XZ (2016) Metabolic reprogramming of alloantigen-activated T cells after hematopoietic cell transplantation. J Clin Invest 126(4):1337–1352. https://doi.org/10.1172/jci82587
28. Ochando J, Ordikhani F, Boros P, Jordan S (2019) The innate immune response to allotransplants: mechanisms and therapeutic potentials. Cell Mol Immunol 16(4):350–356. https://doi.org/10.1038/s41423-019-0216-2
29. Salvadori M, Rosso G, Bertoni E (2015) Update on ischemia-reperfusion injury in kidney transplantation: pathogenesis and treatment. World J Transplant 5(2):52–67. https://doi.org/10.5500/wjt.v5.i2.52

30. Tian R, Abel ED (2001) Responses of GLUT4-deficient hearts to ischemia underscore the importance of glycolysis. Circulation 103(24):2961–2966. https://doi.org/10.1161/01.cir.103.24.2961
31. Marelli-Berg FM, Aksentijevic D (2019) Immunometabolic cross-talk in the inflamed heart. Cell Stress 3(8):240–266. https://doi.org/10.15698/cst2019.08.194
32. Miller EJ, Li J, Leng L, McDonald C, Atsumi T, Bucala R, Young LH (2008) Macrophage migration inhibitory factor stimulates AMP-activated protein kinase in the ischaemic heart. Nature 451(7178):578–582. https://doi.org/10.1038/nature06504
33. Davis CK, Jain SA, Bae ON, Majid A, Rajanikant GK (2018) Hypoxia mimetic agents for ischemic stroke. Front Cell Dev Biol 6:175. https://doi.org/10.3389/fcell.2018.00175
34. Zhang Z, Yao L, Yang J, Wang Z, Du G (2018) PI3K/Akt and HIF-1 signaling pathway in hypoxia-ischemia (Review). Mol Med Rep 18(4):3547–3554. https://doi.org/10.3892/mmr.2018.9375
35. Chouchani ET, Pell VR, Gaude E, Aksentijević D, Sundier SY, Robb EL, Logan A, Nadtochiy SM, Ord ENJ, Smith AC, Eyassu F, Shirley R, Hu CH, Dare AJ, James AM, Rogatti S, Hartley RC, Eaton S, Costa ASH, Brookes PS, Davidson SM, Duchen MR, Saeb-Parsy K, Shattock MJ, Robinson AJ, Work LM, Frezza C, Krieg T, Murphy MP (2014) Ischaemic accumulation of succinate controls reperfusion injury through mitochondrial ROS. Nature 515(7527):431–435. https://doi.org/10.1038/nature13909
36. Inserte J, Garcia-Dorado D, Hernando V, Barba I, Soler-Soler J (2006) Ischemic preconditioning prevents calpain-mediated impairment of Na+/K+-ATPase activity during early reperfusion. Cardiovasc Res 70(2):364–373. https://doi.org/10.1016/j.cardiores.2006.02.017
37. Kahn J, Schemmer P (2018) Control of ischemia-reperfusion injury in liver transplantation: potentials for increasing the donor pool. Visc Med 34(6):444–448. https://doi.org/10.1159/000493889
38. Martins RM, Teodoro JS, Furtado E, Rolo AP, Palmeira CM, Tralhão JG (2018) Recent insights into mitochondrial targeting strategies in liver transplantation. Int J Med Sci 15(3):248–256. https://doi.org/10.7150/ijms.22891
39. Kozlov AV, Lancaster JR Jr, Meszaros AT, Weidinger A (2017) Mitochondria-meditated pathways of organ failure upon inflammation. Redox Biol 13:170–181. https://doi.org/10.1016/j.redox.2017.05.017
40. Zhou H, Zhu P, Wang J, Zhu H, Ren J, Chen Y (2018) Pathogenesis of cardiac ischemia reperfusion injury is associated with CK2α-disturbed mitochondrial homeostasis via suppression of FUNDC1-related mitophagy. Cell Death Differ 25(6):1080–1093. https://doi.org/10.1038/s41418-018-0086-7
41. Lakkis FG, Li XC (2018) Innate allorecognition by monocytic cells and its role in graft rejection. Am J Transplant 18(2):289–292. https://doi.org/10.1111/ajt.14436
42. Lin CM, Gill RG (2016) Direct and indirect allograft recognition: pathways dictating graft rejection mechanisms. Curr Opin Organ Transplant 21(1):40–44. https://doi.org/10.1097/mot.0000000000000263
43. Priyadharshini B, Turka LA (2015) T-cell energy metabolism as a controller of cell fate in transplantation. Curr Opin Organ Transplant 20(1):21–28. https://doi.org/10.1097/mot.0000000000000149
44. da Silva MB, da Cunha FF, Terra FF, Camara NO (2017) Old game, new players: linking classical theories to new trends in transplant immunology. World J Transplant 7(1):1–25. https://doi.org/10.5500/wjt.v7.i1.1
45. Thwe PM, Pelgrom LR, Cooper R, Beauchamp S, Reisz JA, D'Alessandro A, Everts B, Amiel E (2019) Cell-Intrinsic glycogen metabolism supports early glycolytic reprogramming required for dendritic cell immune responses. Cell Metab 30(1):225. https://doi.org/10.1016/j.cmet.2019.05.017
46. Svajger U, Obermajer N, Jeras M (2010) Dendritic cells treated with resveratrol during differentiation from monocytes gain substantial tolerogenic properties upon activation. Immunology 129(4):525–535. https://doi.org/10.1111/j.1365-2567.2009.03205.x

47. Liu Z, Fan H, Jiang S (2013) CD4(+) T-cell subsets in transplantation. Immunol Rev 252(1):183–191. https://doi.org/10.1111/imr.12038
48. Chai H, Yang L, Gao L, Guo Y, Li H, Fan X, Wu B, Xue S, Cai Y, Jiang X, Qin B, Zhang S, Ke Y (2014) Decreased percentages of regulatory T cells are necessary to activate Th1-Th17-Th22 responses during acute rejection of the peripheral nerve xenotransplantation in mice. Transplantation 98(7):729–737. https://doi.org/10.1097/tp.0000000000000319
49. Hall BM (2016) CD4+CD25+ T regulatory cells in transplantation tolerance: 25 years on. Transplantation 100(12):2533–2547. https://doi.org/10.1097/tp.0000000000001436
50. Xu X, Huang H, Wang Q, Cai M, Qian Y, Han Y, Wang X, Gao Y, Yuan M, Xu L, Yao C, Xiao L, Shi B (2017) IFN-γ-producing Th1-like regulatory T cells may limit acute cellular renal allograft rejection: Paradoxical post-transplantation effects of IFN-γ. Immunobiology 222(2):280–290. https://doi.org/10.1016/j.imbio.2016.09.012
51. Gerriets VA, Kishton RJ, Nichols AG, Macintyre AN, Inoue M, Ilkayeva O, Winter PS, Liu X, Priyadharshini B, Slawinska ME, Haeberli L, Huck C, Turka LA, Wood KC, Hale LP, Smith PA, Schneider MA, MacIver NJ, Locasale JW, Newgard CB, Shinohara ML, Rathmell JC (2015) Metabolic programming and PDHK1 control CD4+ T cell subsets and inflammation. J Clin Invest 125(1):194–207. https://doi.org/10.1172/jci76012
52. Shan J, Jin H, Xu Y (2020) T cell metabolism: a new perspective on Th17/treg cell imbalance in systemic lupus erythematosus. Front Immunol 11(1027). https://doi.org/10.3389/fimmu.2020.01027
53. Xu T, Stewart KM, Wang X, Liu K, Xie M, Ryu JK, Li K, Ma T, Wang H, Ni L, Zhu S, Cao N, Zhu D, Zhang Y, Akassoglou K, Dong C, Driggers EM, Ding S (2017) Metabolic control of T(H)17 and induced T(reg) cell balance by an epigenetic mechanism. Nature 548(7666):228–233. https://doi.org/10.1038/nature23475
54. Saxton RA, Sabatini DM (2017) mTOR signaling in growth, metabolism, and disease. Cell 168(6):960–976. https://doi.org/10.1016/j.cell.2017.02.004
55. Siu JHY, Surendrakumar V, Richards JA, Pettigrew GJ (2018) T cell allorecognition pathways in solid organ transplantation. Front Immunol 9:2548. https://doi.org/10.3389/fimmu.2018.02548
56. Azzi JR, Sayegh MH, Mallat SG (2013) Calcineurin inhibitors: 40 years later, can't live without. J Immunol 191(12):5785–5791. https://doi.org/10.4049/jimmunol.1390055
57. Dang EV, Barbi J, Yang HY, Jinasena D, Yu H, Zheng Y, Bordman Z, Fu J, Kim Y, Yen HR, Luo W, Zeller K, Shimoda L, Topalian SL, Semenza GL, Dang CV, Pardoll DM, Pan F (2011) Control of T(H)17/T(reg) balance by hypoxia-inducible factor 1. Cell 146(5):772–784. https://doi.org/10.1016/j.cell.2011.07.033
58. Kurebayashi Y, Nagai S, Ikejiri A, Ohtani M, Ichiyama K, Baba Y, Yamada T, Egami S, Hoshii T, Hirao A, Matsuda S, Koyasu S (2012) PI3K-Akt-mTORC1-S6K1/2 axis controls Th17 differentiation by regulating Gfi1 expression and nuclear translocation of RORgamma. Cell Rep 1(4):360–373. https://doi.org/10.1016/j.celrep.2012.02.007
59. Shi LZ, Wang R, Huang G, Vogel P, Neale G, Green DR, Chi H (2011) HIF1alpha-dependent glycolytic pathway orchestrates a metabolic checkpoint for the differentiation of TH17 and Treg cells. J Exp Med 208(7):1367–1376. https://doi.org/10.1084/jem.20110278
60. Rockey DC, Bell PD, Hill JA (2015) Fibrosis–a common pathway to organ injury and failure. N Engl J Med 372(12):1138–1149. https://doi.org/10.1056/NEJMra1300575
61. Li T, Zhang Z, Bartolacci JG, Dwyer GK, Liu Q, Mathews L, Velayutham M, Roessing A, Lee YC, Dai H, Shiva S, Oberbarnscheidt MH, Dziki JL, Mullett SJ, Wendell SG, Wilkinson JD, Webber SA, Wood-Trageser MA, Watkins SC, Demetris AJ, Hussey GS, Badylak SF, Turnquist HR (2020) Graft IL-33 regulates infiltrating macrophages to protect against chronic rejection. J Clin Invest. https://doi.org/10.1172/jci133008
62. Mills EL, Kelly B, Logan A, Costa ASH, Varma M, Bryant CE, Tourlomousis P, Däbritz JHM, Gottlieb E, Latorre I, Corr SC, McManus G, Ryan D, Jacobs HT, Szibor M, Xavier RJ, Braun T, Frezza C, Murphy MP, O'Neill LA (2016) Succinate dehydrogenase supports metabolic repurposing of mitochondria to drive inflammatory macrophages. Cell 167(2):457-470.e413. https://doi.org/10.1016/j.cell.2016.08.064

63. Boucault L, Séverine B, Ossart J, Guillonneau C (2016) Tolerance in organ transplantation. frontiers-in-transplantology, Chap 3. https://doi.org/10.5772/62653
64. Mondanelli G, Bianchi R, Pallotta MT, Orabona C, Albini E, Iacono A, Belladonna ML, Vacca C, Fallarino F, Macchiarulo A, Ugel S, Bronte V, Gevi F, Zolla L, Verhaar A, Peppelenbosch M, Mazza EMC, Bicciato S, Laouar Y, Santambrogio L, Puccetti P, Volpi C, Grohmann U (2017) A relay pathway between arginine and tryptophan metabolism confers immunosuppressive properties on dendritic cells. Immunity 46(2):233–244. https://doi.org/10.1016/j.immuni.2017.01.005
65. Lagouge M, Argmann C, Gerhart-Hines Z, Meziane H, Lerin C, Daussin F, Messadeq N, Milne J, Lambert P, Elliott P, Geny B, Laakso M, Puigserver P, Auwerx J (2006) Resveratrol improves mitochondrial function and protects against metabolic disease by activating SIRT1 and PGC-1alpha. Cell 127(6):1109–1122. https://doi.org/10.1016/j.cell.2006.11.013
66. Macdougall CE, Wood EG, Loschko J, Scagliotti V, Cassidy FC, Robinson ME, Feldhahn N, Castellano L, Voisin MB, Marelli-Berg F, Gaston-Massuet C, Charalambous M, Longhi MP (2018) Visceral adipose tissue immune homeostasis is regulated by the crosstalk between adipocytes and dendritic cell subsets. Cell Metab 27(3):588-601.e584. https://doi.org/10.1016/j.cmet.2018.02.007
67. Zhao F, Xiao C, Evans KS, Theivanthiran T, DeVito N, Holtzhausen A, Liu J, Liu X, Boczkowski D, Nair S, Locasale JW, Hanks BA (2018) Paracrine Wnt5a-β-catenin signaling triggers a metabolic program that drives dendritic cell tolerization. Immunity 48(1):147-160.e147. https://doi.org/10.1016/j.immuni.2017.12.004
68. Chen Y, Li D, Tsang JY, Niu N, Peng J, Zhu J, Hui K, Xu A, Lui VC, Lamb JR, Tam PK (2011) PPAR-γ signaling and IL-5 inhibition together prevent chronic rejection of MHC Class II-mismatched cardiac grafts. J Heart Lung Transplant 30(6):698–706. https://doi.org/10.1016/j.healun.2011.01.704
69. Zhu HC, Qiu T, Liu XH, Dong WC, Weng XD, Hu CH, Kuang YL, Gao RH, Dan C, Tao T (2012) Tolerogenic dendritic cells generated by RelB silencing using shRNA prevent acute rejection. Cell Immunol 274(1–2):12–18. https://doi.org/10.1016/j.cellimm.2012.02.012
70. Adorini L, Penna G, Giarratana N, Uskokovic M (2003) Tolerogenic dendritic cells induced by vitamin D receptor ligands enhance regulatory T cells inhibiting allograft rejection and autoimmune diseases. J Cell Biochem 88(2):227–233. https://doi.org/10.1002/jcb.10340
71. Liu GY, Sabatini DM (2020) mTOR at the nexus of nutrition, growth, ageing and disease. Nat Rev Mol Cell Biol 21(4):183–203. https://doi.org/10.1038/s41580-019-0199-y
72. Sabatini DM (2017) Twenty-five years of mTOR: uncovering the link from nutrients to growth. Proc Natl Acad Sci U S A 114(45):11818–11825. https://doi.org/10.1073/pnas.1716173114
73. Chung BH, Oh HJ, Piao SG, Sun IO, Kang SH, Choi SR, Park HS, Choi BS, Choi YJ, Park CW, Kim YS, Cho ML, Yang CW (2011) Higher infiltration by Th17 cells compared with regulatory T cells is associated with severe acute T-cell-mediated graft rejection. Exp Mol Med 43(11):630–637. https://doi.org/10.3858/emm.2011.43.11.071
74. Holzknecht ZE, Platt JL (2000) The fine cytokine line between graft acceptance and rejection. Nat Med 6(5):497–498. https://doi.org/10.1038/74963
75. Andreux PA, Houtkooper RH, Auwerx J (2013) Pharmacological approaches to restore mitochondrial function. Nat Rev Drug Discov 12(6):465–483. https://doi.org/10.1038/nrd4023
76. Austin S, St-Pierre J (2012) PGC1α and mitochondrial metabolism–emerging concepts and relevance in ageing and neurodegenerative disorders. J Cell Sci 125(Pt 21):4963–4971. https://doi.org/10.1242/jcs.113662
77. Breda CNS, Davanzo GG, Basso PJ, Saraiva Câmara NO, Moraes-Vieira PMM (2019) Mitochondria as central hub of the immune system. Redox Biol 26. https://doi.org/10.1016/j.redox.2019.101255
78. Finck BN, Kelly DP (2006) PGC-1 coactivators: inducible regulators of energy metabolism in health and disease. J Clin Invest 116(3):615–622. https://doi.org/10.1172/jci27794
79. Klotz L, Dani I, Edenhofer F, Nolden L, Evert B, Paul B, Kolanus W, Klockgether T, Knolle P, Diehl L (2007) Peroxisome proliferator-activated receptor gamma control of dendritic cell

function contributes to development of CD4+ T cell anergy. J Immunol 178(4):2122–2131. https://doi.org/10.4049/jimmunol.178.4.2122
80. Weigt SS, Palchevskiy V, Belperio JA (2017) Inflammasomes and IL-1 biology in the pathogenesis of allograft dysfunction. J Clin Invest 127(6):2022–2029. https://doi.org/10.1172/jci93537
81. de Leur K, Dieterich M, Hesselink DA, Corneth OBJ, Dor F, de Graav GN, Peeters AMA, Mulder A, Kimenai H, Claas FHJ, Clahsen-van Groningen MC, van der Laan LJW, Hendriks RW, Baan CC (2019) Characterization of donor and recipient CD8+ tissue-resident memory T cells in transplant nephrectomies. Sci Rep 9(1):5984. https://doi.org/10.1038/s41598-019-42401-9
82. Kim KW, Kim BM, Doh KC, Cho ML, Yang CW, Chung BH (2018) Clinical significance of CCR7(+)CD8(+) T cells in kidney transplant recipients with allograft rejection. Sci Rep 8(1):8827. https://doi.org/10.1038/s41598-018-27141-6
83. Mothe-Satney I, Murdaca J, Sibille B, Rousseau AS, Squillace R, Le Menn G, Rekima A, Larbret F, Pelé J, Verhasselt V, Grimaldi PA, Neels JG (2016) A role for peroxisome proliferator-activated receptor beta in T cell development. Sci Rep 6:34317. https://doi.org/10.1038/srep34317
84. Scharping NE, Menk AV, Moreci RS, Whetstone RD, Dadey RE, Watkins SC, Ferris RL, Delgoffe GM (2016) The tumor microenvironment represses T cell mitochondrial biogenesis to drive intratumoral T cell metabolic insufficiency and dysfunction. Immunity 45(2):374–388. https://doi.org/10.1016/j.immuni.2016.07.009
85. Ahmadian M, Suh JM, Hah N, Liddle C, Atkins AR, Downes M, Evans RM (2013) PPARγ signaling and metabolism: the good, the bad and the future. Nat Med 19(5):557–566. https://doi.org/10.1038/nm.3159
86. Michelet X, Dyck L, Hogan A, Loftus RM, Duquette D, Wei K, Beyaz S, Tavakkoli A, Foley C, Donnelly R, O'Farrelly C, Raverdeau M, Vernon A, Pettee W, O'Shea D, Nikolajczyk BS, Mills KHG, Brenner MB, Finlay D, Lynch L (2018) Metabolic reprogramming of natural killer cells in obesity limits antitumor responses. Nat Immunol 19(12):1330–1340. https://doi.org/10.1038/s41590-018-0251-7
87. Wahli W, Michalik L (2012) PPARs at the crossroads of lipid signaling and inflammation. Trends Endocrinol Metab 23(7):351–363. https://doi.org/10.1016/j.tem.2012.05.001
88. Pearce EJ, Everts B (2015) Dendritic cell metabolism. Nat Rev Immunol 15(1):18–29. https://doi.org/10.1038/nri3771
89. Zhuang Q, Liu Q, Divito SJ, Zeng Q, Yatim KM, Hughes AD, Rojas-Canales DM, Nakao A, Shufesky WJ, Williams AL, Humar R, Hoffman RA, Shlomchik WD, Oberbarnscheidt MH, Lakkis FG, Morelli AE (2016) Graft-infiltrating host dendritic cells play a key role in organ transplant rejection. Nat Commun 7:12623. https://doi.org/10.1038/ncomms12623
90. Park H-J, Kim D-H, Choi J-Y, Kim W-J, Kim JY, Senejani AG, Hwang SS, Kim LK, Tobiasova Z, Lee GR, Craft J, Bothwell ALM, Choi J-M (2014) PPARγ negatively regulates T cell activation to prevent follicular helper T cells and germinal center formation. PLoS ONE 9(6):e99127–e99127. https://doi.org/10.1371/journal.pone.0099127
91. Choi JM, Bothwell AL (2012) The nuclear receptor PPARs as important regulators of T-cell functions and autoimmune diseases. Mol Cells 33(3):217–222. https://doi.org/10.1007/s10059-012-2297-y
92. Lee JW, Ko J, Ju C, Eltzschig HK (2019) Hypoxia signaling in human diseases and therapeutic targets. Exp Mol Med 51(6):1–13. https://doi.org/10.1038/s12276-019-0235-1
93. Guak H, Al Habyan S, Ma EH, Aldossary H, Al-Masri M, Won SY, Ying T, Fixman ED, Jones RG, McCaffrey LM, Krawczyk CM (2018) Glycolytic metabolism is essential for CCR7 oligomerization and dendritic cell migration. Nat Commun 9(1):2463. https://doi.org/10.1038/s41467-018-04804-6
94. Bernhardt WM, Gottmann U, Doyon F, Buchholz B, Campean V, Schödel J, Reisenbuechler A, Klaus S, Arend M, Flippin L, Willam C, Wiesener MS, Yard B, Warnecke C, Eckardt KU (2009) Donor treatment with a PHD-inhibitor activating HIFs prevents graft injury and prolongs survival in an allogenic kidney transplant model. Proc Natl Acad Sci USA 106(50):21276–21281. https://doi.org/10.1073/pnas.0903978106

95. Conde E, Giménez-Moyano S, Martín-Gómez L, Rodríguez M, Ramos ME, Aguado-Fraile E, Blanco-Sanchez I, Saiz A, García-Bermejo ML (2017) HIF-1α induction during reperfusion avoids maladaptive repair after renal ischemia/reperfusion involving miR127-3p. Sci Rep 7(1):41099. https://doi.org/10.1038/srep41099
96. Schödel J, Ratcliffe PJ (2019) Mechanisms of hypoxia signalling: new implications for nephrology. Nat Rev Nephrol 15(10):641–659. https://doi.org/10.1038/s41581-019-0182-z
97. Parbin S, Kar S, Shilpi A, Sengupta D, Deb M, Rath SK, Patra SK (2014) Histone deacetylases: a saga of perturbed acetylation homeostasis in cancer. J Histochem Cytochem 62(1):11–33. https://doi.org/10.1369/0022155413506582
98. Kupis W, Pałyga J, Tomal E, Niewiadomska E (2016) The role of sirtuins in cellular homeostasis. J Physiol Biochem 72(3):371–380. https://doi.org/10.1007/s13105-016-0492-6
99. Hayden MS, West AP, Ghosh S (2006) NF-kappaB and the immune response. Oncogene 25(51):6758–6780. https://doi.org/10.1038/sj.onc.1209943
100. Imai S, Guarente L (2014) NAD+ and sirtuins in aging and disease. Trends Cell Biol 24(8):464–471. https://doi.org/10.1016/j.tcb.2014.04.002
101. Koyama T, Kume S, Koya D, Araki S, Isshiki K, Chin-Kanasaki M, Sugimoto T, Haneda M, Sugaya T, Kashiwagi A, Maegawa H, Uzu T (2011) SIRT3 attenuates palmitate-induced ROS production and inflammation in proximal tubular cells. Free Radic Biol Med 51(6):1258–1267. https://doi.org/10.1016/j.freeradbiomed.2011.05.028
102. Yeung F, Hoberg JE, Ramsey CS, Keller MD, Jones DR, Frye RA, Mayo MW (2004) Modulation of NF-kappaB-dependent transcription and cell survival by the SIRT1 deacetylase. Embo j 23(12):2369–2380. https://doi.org/10.1038/sj.emboj.7600244
103. Giralt A, Villarroya F (2012) SIRT3, a pivotal actor in mitochondrial functions: metabolism, cell death and aging. Biochem J 444(1):1–10. https://doi.org/10.1042/bj20120030
104. Zhou Q, Lv D, Xia Y, Zhao Z, Zou H (2018) Decreased expression of sirtuin 3 protein correlates with early stage chronic renal allograft dysfunction in a rat kidney model. Exp Ther Med 15(4):3725–3732. https://doi.org/10.3892/etm.2018.5909
105. Elbe H, Vardi N, Esrefoglu M, Ates B, Yologlu S, Taskapan C (2015) Amelioration of streptozotocin-induced diabetic nephropathy by melatonin, quercetin, and resveratrol in rats. Hum Exp Toxicol 34(1):100–113. https://doi.org/10.1177/0960327114531995
106. Hasegawa K, Wakino S, Simic P, Sakamaki Y, Minakuchi H, Fujimura K, Hosoya K, Komatsu M, Kaneko Y, Kanda T, Kubota E, Tokuyama H, Hayashi K, Guarente L, Itoh H (2013) Renal tubular Sirt1 attenuates diabetic albuminuria by epigenetically suppressing Claudin-1 overexpression in podocytes. Nat Med 19(11):1496–1504. https://doi.org/10.1038/nm.3363
107. Huang K, Huang J, Xie X, Wang S, Chen C, Shen X, Liu P, Huang H (2013) Sirt1 resists advanced glycation end products-induced expressions of fibronectin and TGF-β1 by activating the Nrf2/ARE pathway in glomerular mesangial cells. Free Radic Biol Med 65:528–540. https://doi.org/10.1016/j.freeradbiomed.2013.07.029
108. Wen D, Huang X, Zhang M, Zhang L, Chen J, Gu Y, Hao CM (2013) Resveratrol attenuates diabetic nephropathy via modulating angiogenesis. PLoS ONE 8(12). https://doi.org/10.1371/journal.pone.0082336
109. Xu F, Wang Y, Cui W, Yuan H, Sun J, Wu M, Guo Q, Kong L, Wu H, Miao L (2014) Resveratrol prevention of diabetic nephropathy is associated with the suppression of renal inflammation and mesangial cell proliferation: possible roles of Akt/NF-κB pathway. Int J Endocrinol 2014. https://doi.org/10.1155/2014/289327
110. Gao R, Chen J, Hu Y, Li Z, Wang S, Shetty S, Fu J (2014) Sirt1 deletion leads to enhanced inflammation and aggravates endotoxin-induced acute kidney injury. PLoS ONE 9(6). https://doi.org/10.1371/journal.pone.0098909
111. Hu M, Wang C, Zhang GY, Saito M, Wang YM, Fernandez MA, Wang Y, Wu H, Hawthorne WJ, Jones C, O'Connell PJ, Sparwasser T, Bishop GA, Sharland AF, Alexander SI (2013) Infiltrating Foxp3(+) regulatory T cells from spontaneously tolerant kidney allografts demonstrate donor-specific tolerance. Am J Transplant 13(11):2819–2830. https://doi.org/10.1111/ajt.12445

112. Levine MH, Wang Z, Xiao H, Jiao J, Wang L, Bhatti TR, Hancock WW, Beier UH (2016) Targeting Sirtuin-1 prolongs murine renal allograft survival and function. Kidney Int 89(5):1016–1026. https://doi.org/10.1016/j.kint.2015.12.051
113. Zhang J, Lee SM, Shannon S, Gao B, Chen W, Chen A, Divekar R, McBurney MW, Braley-Mullen H, Zaghouani H, Fang D (2009) The type III histone deacetylase Sirt1 is essential for maintenance of T cell tolerance in mice. J Clin Invest 119(10):3048–3058. https://doi.org/10.1172/jci38902
114. Lim HW, Kang SG, Ryu JK, Schilling B, Fei M, Lee IS, Kehasse A, Shirakawa K, Yokoyama M, Schnölzer M, Kasler HG, Kwon HS, Gibson BW, Sato H, Akassoglou K, Xiao C, Littman DR, Ott M, Verdin E (2015) SIRT1 deacetylates RORγt and enhances Th17 cell generation. J Exp Med 212(5):607–617. https://doi.org/10.1084/jem.20132378
115. Kwon HS, Lim HW, Wu J, Schnölzer M, Verdin E, Ott M (2012) Three novel acetylation sites in the Foxp3 transcription factor regulate the suppressive activity of regulatory T cells. J Immunol 188(6):2712–2721. https://doi.org/10.4049/jimmunol.1100903
116. Kong S, McBurney MW, Fang D (2012) Sirtuin 1 in immune regulation and autoimmunity. Immunol Cell Biol 90(1):6–13. https://doi.org/10.1038/icb.2011.102
117. Fairchild RL (2016) Juicing Tregs in situ to improve kidney allograft outcomes. Kidney Int 89(5):976–978. https://doi.org/10.1016/j.kint.2016.01.020
118. Akimova T, Xiao H, Liu Y, Bhatti TR, Jiao J, Eruslanov E, Singhal S, Wang L, Han R, Zacharia K, Hancock WW, Beier UH (2014) Targeting sirtuin-1 alleviates experimental autoimmune colitis by induction of Foxp3+ T-regulatory cells. Mucosal Immunol 7(5):1209–1220. https://doi.org/10.1038/mi.2014.10
119. Beier UH, Akimova T, Liu Y, Wang L, Hancock WW (2011) Histone/protein deacetylases control Foxp3 expression and the heat shock response of T-regulatory cells. Curr Opin Immunol 23(5):670–678. https://doi.org/10.1016/j.coi.2011.07.002
120. Beier UH, Wang L, Han R, Akimova T, Liu Y, Hancock WW (2012) Histone deacetylases 6 and 9 and sirtuin-1 control Foxp3+ regulatory T cell function through shared and isoform-specific mechanisms. Sci Signal 5(229):ra45. https://doi.org/10.1126/scisignal.2002873
121. Beier UH, Wang L, Bhatti TR, Liu Y, Han R, Ge G, Hancock WW (2011) Sirtuin-1 targeting promotes Foxp3+ T-regulatory cell function and prolongs allograft survival. Mol Cell Biol 31(5):1022–1029. https://doi.org/10.1128/mcb.01206-10
122. Tao R, Hancock WW (2008) Resistance of Foxp3+ regulatory T cells to Nur77-induced apoptosis promotes allograft survival. PLoS ONE 3(5). https://doi.org/10.1371/journal.pone.0002321
123. Miyajima M, Chase CM, Alessandrini A, Farkash EA, Della Pelle P, Benichou G, Graham JA, Madsen JC, Russell PS, Colvin RB (2011) Early acceptance of renal allografts in mice is dependent on foxp3(+) cells. Am J Pathol 178(4):1635–1645. https://doi.org/10.1016/j.ajpath.2010.12.024
124. Liu P, Huang G, Wei T, Gao J, Huang C, Sun M, Zhu L (1864) Shen W (2018) Sirtuin 3-induced macrophage autophagy in regulating NLRP3 inflammasome activation. Biochim Biophys Acta Mol Basis Dis 3:764–777. https://doi.org/10.1016/j.bbadis.2017.12.027
125. Toubai T, Tamaki H, Peltier DC, Rossi C, Oravecz-Wilson K, Liu C, Zajac C, Wu J, Sun Y, Fujiwara H, Henig I, Kim S, Lombard DB, Reddy P (2018) Mitochondrial deacetylase SIRT3 plays an important role in donor T cell responses after experimental allogeneic hematopoietic transplantation. J Immunol 201(11):3443–3455. https://doi.org/10.4049/jimmunol.1800148
126. Netea MG, Domínguez-Andrés J, Barreiro LB, Chavakis T, Divangahi M, Fuchs E, Joosten LAB, van der Meer JWM, Mhlanga MM, Mulder WJM, Riksen NP, Schlitzer A, Schultze JL, Stabell Benn C, Sun JC, Xavier RJ, Latz E (2020) Defining trained immunity and its role in health and disease. Nat Rev Immunol 20(6):375–388. https://doi.org/10.1038/s41577-020-0285-6
127. Domínguez-Andrés J, Joosten LA, Netea MG (2019) Induction of innate immune memory: the role of cellular metabolism. Curr Opin Immunol 56:10–16. https://doi.org/10.1016/j.coi.2018.09.001

128. Norata GD, Caligiuri G, Chavakis T, Matarese G, Netea MG, Nicoletti A, O'Neill LA, Marelli-Berg FM (2015) The cellular and molecular basis of translational immunometabolism. Immunity 43(3):421–434. https://doi.org/10.1016/j.immuni.2015.08.023
129. Penkov S, Mitroulis I, Hajishengallis G, Chavakis T (2019) Immunometabolic crosstalk: an ancestral principle of trained immunity? Trends Immunol 40(1):1–11. https://doi.org/10.1016/j.it.2018.11.002
130. Cheng SC, Quintin J, Cramer RA, Shepardson KM, Saeed S, Kumar V, Giamarellos-Bourboulis EJ, Martens JH, Rao NA, Aghajanirefah A, Manjeri GR, Li Y, Ifrim DC, Arts RJ, van der Veer BM, Deen PM, Logie C, O'Neill LA, Willems P, van de Veerdonk FL, van der Meer JW, Ng A, Joosten LA, Wijmenga C, Stunnenberg HG, Xavier RJ, Netea MG (2014) mTOR- and HIF-1α-mediated aerobic glycolysis as metabolic basis for trained immunity. Science 345(6204):1250684. https://doi.org/10.1126/science.1250684
131. Donohoe DR, Bultman SJ (2012) Metaboloepigenetics: interrelationships between energy metabolism and epigenetic control of gene expression. J Cell Physiol 227(9):3169–3177. https://doi.org/10.1002/jcp.24054
132. Cordes T, Wallace M, Michelucci A, Divakaruni AS, Sapcariu SC, Sousa C, Koseki H, Cabrales P, Murphy AN, Hiller K, Metallo CM (2016) Immunoresponsive gene 1 and itaconate inhibit succinate dehydrogenase to modulate intracellular succinate levels. J Biol Chem 291(27):14274–14284. https://doi.org/10.1074/jbc.M115.685792
133. Lampropoulou V, Sergushichev A, Bambouskova M, Nair S, Vincent EE, Loginicheva E, Cervantes-Barragan L, Ma X, Huang SC, Griss T, Weinheimer CJ, Khader S, Randolph GJ, Pearce EJ, Jones RG, Diwan A, Diamond MS, Artyomov MN (2016) Itaconate links inhibition of succinate dehydrogenase with macrophage metabolic remodeling and regulation of inflammation. Cell Metab 24(1):158–166. https://doi.org/10.1016/j.cmet.2016.06.004
134. Liu PS, Wang H, Li X, Chao T, Teav T, Christen S, Di Conza G, Cheng WC, Chou CH, Vavakova M, Muret C, Debackere K, Mazzone M, Huang HD, Fendt SM, Ivanisevic J, Ho PC (2017) α-ketoglutarate orchestrates macrophage activation through metabolic and epigenetic reprogramming. Nat Immunol 18(9):985–994. https://doi.org/10.1038/ni.3796
135. Mills EL, Ryan DG, Prag HA, Dikovskaya D, Menon D, Zaslona Z, Jedrychowski MP, Costa ASH, Higgins M, Hams E, Szpyt J, Runtsch MC, King MS, McGouran JF, Fischer R, Kessler BM, McGettrick AF, Hughes MM, Carroll RG, Booty LM, Knatko EV, Meakin PJ, Ashford MLJ, Modis LK, Brunori G, Sévin DC, Fallon PG, Caldwell ST, Kunji ERS, Chouchani ET, Frezza C, Dinkova-Kostova AT, Hartley RC, Murphy MP, O'Neill LA (2018) Itaconate is an anti-inflammatory metabolite that activates Nrf2 via alkylation of KEAP1. Nature 556(7699):113–117. https://doi.org/10.1038/nature25986
136. Bode K, Bujupi F, Link C, Hein T, Zimmermann S, Peiris D, Jaquet V, Lepenies B, Weyd H, Krammer PH (2019) Dectin-1 binding to annexins on apoptotic cells induces peripheral immune tolerance via NADPH oxidase-2. Cell Rep 29(13):4435-4446.e4439. https://doi.org/10.1016/j.celrep.2019.11.086
137. Netea MG, Joosten LA, Latz E, Mills KH, Natoli G, Stunnenberg HG, O'Neill LA, Xavier RJ (2016) Trained immunity: a program of innate immune memory in health and disease. Science 352(6284):aaf1098. https://doi.org/10.1126/science.aaf1098
138. Shen H, Goldstein DR (2009) IL-6 and TNF-alpha synergistically inhibit allograft acceptance. J Am Soc Nephrol 20(5):1032–1040. https://doi.org/10.1681/asn.2008070778
139. Eckes B, Colucci-Guyon E, Smola H, Nodder S, Babinet C, Krieg T, Martin P (2000) Impaired wound healing in embryonic and adult mice lacking vimentin. J Cell Sci 113(Pt 13):2455–2462
140. Thiagarajan PS, Yakubenko VP, Elsori DH, Yadav SP, Willard B, Tan CD, Rodriguez ER, Febbraio M, Cathcart MK (2013) Vimentin is an endogenous ligand for the pattern recognition receptor Dectin-1. Cardiovasc Res 99(3):494–504. https://doi.org/10.1093/cvr/cvt117
141. Azimzadeh AM, Pfeiffer S, Wu GS, Schröder C, Zhou H, Zorn GL 3rd, Kehry M, Miller GG, Rose ML, Pierson RN 3rd (2005) Humoral immunity to vimentin is associated with cardiac allograft injury in nonhuman primates. Am J Transplant 5(10):2349–2359. https://doi.org/10.1111/j.1600-6143.2005.01022.x

142. Braza MS, van Leent MMT, Lameijer M, Sanchez-Gaytan BL, Arts RJW, Pérez-Medina C, Conde P, Garcia MR, Gonzalez-Perez M, Brahmachary M, Fay F, Kluza E, Kossatz S, Dress RJ, Salem F, Rialdi A, Reiner T, Boros P, Strijkers GJ, Calcagno CC, Ginhoux F, Marazzi I, Lutgens E, Nicolaes GAF, Weber C, Swirski FK, Nahrendorf M, Fisher EA, Duivenvoorden R, Fayad ZA, Netea MG, Mulder WJM, Ochando J (2018) Inhibiting inflammation with myeloid cell-specific nanobiologics promotes organ transplant acceptance. Immunity 49(5):819-828.e816. https://doi.org/10.1016/j.immuni.2018.09.008
143. Miller LW, Granville DJ, Narula J, McManus BM (2001) Apoptosis in cardiac transplant rejection. Cardiol Clin 19(1):141–154. https://doi.org/10.1016/s0733-8651(05)70200-9
144. Rose ML (2004) De novo production of antibodies after heart or lung transplantation should be regarded as an early warning system. J Heart Lung Transplant 23(4):385–395. https://doi.org/10.1016/j.healun.2003.08.028
145. Linkermann A, Bräsen JH, Darding M, Jin MK, Sanz AB, Heller JO, De Zen F, Weinlich R, Ortiz A, Walczak H, Weinberg JM, Green DR, Kunzendorf U, Krautwald S (2013) Two independent pathways of regulated necrosis mediate ischemia-reperfusion injury. Proc Natl Acad Sci USA 110(29):12024–12029. https://doi.org/10.1073/pnas.1305538110
146. Huang Y, Yin H, Han J, Huang B, Xu J, Zheng F, Tan Z, Fang M, Rui L, Chen D, Wang S, Zheng X, Wang CY, Gong F (2007) Extracellular hmgb1 functions as an innate immune-mediator implicated in murine cardiac allograft acute rejection. Am J Transplant 7(4):799–808. https://doi.org/10.1111/j.1600-6143.2007.01734.x
147. Scaffidi P, Misteli T, Bianchi ME (2002) Release of chromatin protein HMGB1 by necrotic cells triggers inflammation. Nature 418(6894):191–195. https://doi.org/10.1038/nature00858
148. Wu H, Ma J, Wang P, Corpuz TM, Panchapakesan U, Wyburn KR, Chadban SJ (2010) HMGB1 contributes to kidney ischemia reperfusion injury. J Am Soc Nephrol 21(11):1878–1890. https://doi.org/10.1681/asn.2009101048
149. Girardin SE, Boneca IG, Viala J, Chamaillard M, Labigne A, Thomas G, Philpott DJ, Sansonetti PJ (2003) Nod2 is a general sensor of peptidoglycan through muramyl dipeptide (MDP) detection. J Biol Chem 278(11):8869–8872. https://doi.org/10.1074/jbc.C200651200
150. Sabbah A, Chang TH, Harnack R, Frohlich V, Tominaga K, Dube PH, Xiang Y, Bose S (2009) Activation of innate immune antiviral responses by Nod2. Nat Immunol 10(10):1073–1080. https://doi.org/10.1038/ni.1782
151. Chong AS, Alegre ML (2012) The impact of infection and tissue damage in solid-organ transplantation. Nat Rev Immunol 12(6):459–471. https://doi.org/10.1038/nri3215
152. Martin-Gandul C, Mueller NJ, Pascual M, Manuel O (2015) The impact of infection on chronic allograft dysfunction and allograft survival after solid organ transplantation. Am J Transplant 15(12):3024–3040. https://doi.org/10.1111/ajt.13486
153. Kleinnijenhuis J, Quintin J, Preijers F, Joosten LA, Ifrim DC, Saeed S, Jacobs C, van Loenhout J, de Jong D, Stunnenberg HG, Xavier RJ, van der Meer JW, van Crevel R, Netea MG (2012) Bacille Calmette-Guerin induces NOD2-dependent nonspecific protection from reinfection via epigenetic reprogramming of monocytes. Proc Natl Acad Sci U S A 109(43):17537–17542. https://doi.org/10.1073/pnas.1202870109
154. Mulder WJM, Ochando J, Joosten LAB, Fayad ZA, Netea MG (2019) Therapeutic targeting of trained immunity. Nat Rev Drug Discov 18(7):553–566. https://doi.org/10.1038/s41573-019-0025-4
155. Tabibian JH, Kenderian SS (2017) The microbiome and immune regulation after transplantation. Transplantation 101(1):56–62. https://doi.org/10.1097/tp.0000000000001444
156. Round JL, Mazmanian SK (2009) The gut microbiota shapes intestinal immune responses during health and disease. Nat Rev Immunol 9(5):313–323. https://doi.org/10.1038/nri2515
157. Thaiss CA, Zmora N, Levy M, Elinav E (2016) The microbiome and innate immunity. Nature 535(7610):65–74. https://doi.org/10.1038/nature18847
158. Kim CH (2018) Immune regulation by microbiome metabolites. Immunology 154(2):220–229. https://doi.org/10.1111/imm.12930

159. Quintana FJ, Basso AS, Iglesias AH, Korn T, Farez MF, Bettelli E, Caccamo M, Oukka M, Weiner HL (2008) Control of T(reg) and T(H)17 cell differentiation by the aryl hydrocarbon receptor. Nature 453(7191):65–71. https://doi.org/10.1038/nature06880
160. Goudot C, Coillard A, Villani AC, Gueguen P, Cros A, Sarkizova S, Tang-Huau TL, Bohec M, Baulande S, Hacohen N, Amigorena S, Segura E (2017) Aryl hydrocarbon receptor controls monocyte differentiation into dendritic cells versus macrophages. Immunity 47(3):582-596.e586. https://doi.org/10.1016/j.immuni.2017.08.016
161. Singh NP, Singh UP, Rouse M, Zhang J, Chatterjee S, Nagarkatti PS, Nagarkatti M (2016) Dietary indoles suppress delayed-type hypersensitivity by inducing a switch from proinflammatory Th17 cells to anti-inflammatory regulatory T cells through regulation of microRNA. J Immunol 196(3):1108–1122. https://doi.org/10.4049/jimmunol.1501727
162. Levy M, Thaiss CA, Elinav E (2016) Metabolites: messengers between the microbiota and the immune system. Genes Dev 30(14):1589–1597. https://doi.org/10.1101/gad.284091.116
163. Singh N, Gurav A, Sivaprakasam S, Brady E, Padia R, Shi H, Thangaraju M, Prasad PD, Manicassamy S, Munn DH, Lee JR, Offermanns S, Ganapathy V (2014) Activation of Gpr109a, receptor for niacin and the commensal metabolite butyrate, suppresses colonic inflammation and carcinogenesis. Immunity 40(1):128–139. https://doi.org/10.1016/j.immuni.2013.12.007
164. Haase S, Haghikia A, Wilck N, Müller DN, Linker RA (2018) Impacts of microbiome metabolites on immune regulation and autoimmunity. Immunology 154(2):230–238. https://doi.org/10.1111/imm.12933
165. Nastasi C, Candela M, Bonefeld CM, Geisler C, Hansen M, Krejsgaard T, Biagi E, Andersen MH, Brigidi P, Ødum N, Litman T, Woetmann A (2015) The effect of short-chain fatty acids on human monocyte-derived dendritic cells. Sci Rep 5:16148. https://doi.org/10.1038/srep16148
166. Dery KJ, Kadono K, Hirao H, Górski A, Kupiec-Weglinski JW (2020) Microbiota in organ transplantation: an immunological and therapeutic conundrum? Cell Immunol 351. https://doi.org/10.1016/j.cellimm.2020.104080