Handbook of Experimental Pharmacology 272

Howard J. Eisen *Editor*

Pharmacology of Immunosuppression

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Volume 272

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Pharmacology of Immunosuppression

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Preface

This volume of *The Handbook of Experimental Pharmacology* provides insights into the broad range of immunosuppressive agents used to modulate immune responses against organs of the body. Given the complexity of the immune pathways involved and their interplay, taming the immune system to treat and control a multitude of naturally occurring diseases and diseases that are a consequence of modern life-saving therapies such as solid organ and bone marrow transplantation is truly remarkable. The volume begins with a detailed review of salient immune pathways involved in the alloimmune response as well as immune responses to infections, malignancies, and other threats. The subsequent chapters then discuss how these pathways go awry in patients, resulting in autoimmune diseases, and how these pathways are controlled in these autoimmune diseases (rheumatic and multiple sclerosis) using immunosuppression. A further discussion relates to how immunosuppression improves clinical outcomes in these patients through modulation of the immune system.

A preponderance of the chapters address the most dramatic use of immunosuppression currently which is to blunt the alloimmune onslaught which is a consequence of solid organ and bone marrow allo-transplantation. In these settings, the alloimmune response is a consequence of desperate efforts to control life-threatening diseases such as advanced heart or lung diseases, end-stage kidney and liver diseases, and hematologic malignancies. In these clinical scenarios, organ transplantation is often performed using immunologically different donors, resulting in the trading of one disease, the life-threatening one, which necessitated transplantation with the post-transplant state. Left uncontrolled, the alloimmune responses to organ transplantation can result in the swift destruction of the allograft and death of the recipient. One only need to think of the earliest heart transplant recipients in the late 1960s and early 1970s to fully comprehend both the consequences of not understanding the pathophysiology of the alloimmune responses and the therapies used to control these which have resulted in the modern miracle which is organ transplantation. This lack of understanding of immunology and the lack of effective immunosuppressive drugs also explain why the very earliest renal transplant in Boston in the 1950s was between identical twins. It was not until 1974 that the alloimmune response was deciphered by Doherty and Zinkernagel, ushering in an era of more specific, less toxic immunosuppressive agents. These agents have allowed for the

development of successful, safe transplants which in turn have saved millions. The understanding of how these immunosuppressive can be used in organ transplant recipients clinically has been a dynamic process resulting from the results of clinical trials, experience, and registries. This evolution is detailed and explained in the organ transplant specific chapters. Chapters dealing with pharmacology and immunology ranging from small inhibitory molecules like the calcineurin and mTOR inhibitors which block pivotal parts of the immune cascade to less specific agents like anti-proliferatives which block the proliferation of immune cells to corticosteroids which have more global effects explain how these agents are utilized for immunosuppression and how to optimize their clinical effects. A chapter discussing the use of antibodies to blunt the alloimmune response is also included.

It is well known that managing transplant recipients is a "tightrope" balancing act where either under- or over-immunosuppression can be catastrophic, the former causing allograft failure and the latter causing infections, malignancies, or other complications all of which can be life-threatening. The complications of immunosuppressive agents are delineated into specific categories including infections, malignancies, and metabolic complications. The fact that these complications can occur even with routine use of immunosuppression by experienced clinicians illustrates that the use of immunosuppression to manage autoimmune disease and organ transplantation is a high-stakes game.

The authors of these chapters are experts in their fields with considerable experience in the use of these agents and often leaders in conducting clinical trials of new agents. A chapter is included on novel immunosuppression on the horizon.

This volume provides the scientific underpinning for understanding the mechanism of immunosuppressive agents' actions. The volume also provides information for use of immunosuppressive agents in specific conditions including autoimmune diseases and organ transplants. Finally, the myriad of complications caused by immunosuppression are discussed in detail. This volume will be of considerable interest and use for clinicians, physician-scientists, clinical investigators, residents, fellows, and medical students. I thank the authors who contributed these outstanding chapters. They are experts and leaders in their fields. Their work can be considered authoritative sources in their fields.

Hershey, PA, USA Howard J. Eisen

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The Biology and Molecular Basis of Organ Transplant Rejection

Philip F. Halloran, Gunilla Einecke, Majid L. N. Sikosana, and Katelynn Madill-Thomsen

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Abstract

Allograft rejection is defined as tissue injury in a transplanted allogeneic organ produced by the effector mechanisms of the adaptive alloimmune response. Effector T lymphocytes and IgG alloantibodies cause two different types of rejection that can occur either individually or simultaneously: T cell-mediated rejection (TCMR) and antibody-mediated rejection (ABMR). In TCMR, cognate effector T cells infiltrate the graft and orchestrate an interstitial inflammatory response in the kidney interstitium in which effector T cells engage antigen-presenting myeloid cells, activating the T cells, antigen-presenting cells, and macrophages. The result is intense expression of IFNG and IFNG-induced molecules, expression of effector T cell molecules and macrophage molecules and checkpoints, and deterioration of parenchymal function. The diagnostic lesions of TCMR follow, i.e. interstitial inflammation, parenchymal deterioration, and intimal arteritis. In ABMR, HLA IgG alloantibodies produced by plasma cells bind to the donor antigens on graft microcirculation, leading to complement activation, margination, and activation of NK cells and neutrophils and monocytes, and endothelial injury, sometimes with intimal arteritis. TCMR becomes infrequent after 5–10 years post-transplant, probably reflecting adaptive mechanisms such as checkpoints, but ABMR can present even decades post-transplant. Some rejection is triggered by inadequate immunosuppression and non-adherence, challenging the clinician to target effective immunosuppression even decades post-transplant.

Keywords

Alloimmune response · Antibody-mediated rejection · Donor-specific antibody · Organ transplantation · Rejection · T cell-mediated rejection

1 Introduction and Overview

This chapter focuses on organ transplant (allograft) rejection, with a particular focus on kidney and heart transplants, but we will also consider the effects of parenchymal injury. T cells with alpha-beta receptors (TCRs) recognizing major histocompatibility complex (MHC – human, HLA) proteins are essential for all graft rejection: animals with no thymus and no T cells cannot reject organ allografts. For reviews, see Halloran (2004); Halloran et al. (2016a). Aspects of the rejection process have been covered in previous reviews (Einecke and Halloran 2007).

Much new information about rejection included here has been generated in the development of the Molecular Microscope Diagnostic System (MMDx) (Halloran

et al. 2018) for organ transplants biopsies, including kidney (Reeve et al. 2017), heart (Parkes et al. 2019), lung transbronchial biopsies (Halloran et al. 2019), lung mucosal biopsies (Halloran et al. 2020), and liver biopsies (Madill-Thomsen et al. 2020).

Much of what we know about rejection comes from kidney transplant studies because the core biopsies are abundant and more easily read than the more challenging heart and lung biopsies. This chapter will often refer to kidney studies but most lessons are generalizable to other organ transplants.

It is necessary to understand tissue injury, which is universal in donation and implantation of organ transplants and may help activate antigen presentation and adaptive immune responses. Nonimmune and immune injury is additive. However, injury is probably not necessary for activating the immune response: some "ticking over" of the antigen presentation system may always be occurring.

1.1 Structure and Function of the Immune System and Some Molecules to Know

We cannot cover the molecular biology of all elements of adaptive and innate immunity (inflammation) in this chapter, and the reader is encouraged to have some familiarity with the development of the adaptive immune system; the lymphoid organs; the key antigen recognition molecules – T cell receptors, B cell receptors and immunoglobulins; the major histocompatibility complex proteins (in humans, the HLA complex), the cytokines and the chemokines; and the mechanisms of inflammation.

T cells are generated in the thymus from marrow precursors, rearranging their TCR genes, expressing TCRs, and undergoing positive and negative selection. B cells arise from marrow precursors in the bone marrow, rearranging their immunoglobulin light and heavy chain genes, and undergo negative selection. Mature T and B cells then populate the secondary lymphoid organs.

Organ transplantation between genetically non-identical humans leads to the activation of a large number of alloreactive clones of T and B lymphocytes that specifically recognize the mismatched donor alloantigens and can generate antigen-specific effector functions leading to the destruction of the transplant. Alloantigens are antigenic differences between individuals controlled by polymorphic gene differences. MHC alloantigens are of two classes – class I (specialized to engage CD8 T cells) and class II – specialized to engage CD4 T cells. All contain peptides in their groove between alpha-helices. Each can be "seen" by T cells in three ways: as intact donor molecules (direct) recognition); as peptides in host MHC molecules (indirect); or as intact donor molecules on islands on the membrane from donor cells that have been acquired by host cells (semi-direct"). Thus MHC alloantigens are peptide-MHC complexes that present non-self features, due to either non-self (donor) amino acid sequences in the MHC protein itself, non-self donor MHC peptides in MHC grooves, or both. B cell alloantigens (that will generate alloantibodies) are intact non-self MHC proteins; the peptide is usually not relevant.

The two cognate (i.e., antigen-specific) effector systems of the adaptive immune response generated during the alloimmune response are the effector T lymphocytes, which cause T cell-mediated rejection (TCMR) and IgG alloantibodies, which cause antibody-mediated rejection (ABMR). Under usual immunosuppressive regimens (Halloran 2004), clinical rejection episodes take characteristic TCMR and ABMR and mixed forms and are diagnosed by biopsies read by histology and molecular platforms. The possibility of NK cell recognition of "missing self" must also be considered within the syndrome of ABMR phenotypes (Callemeyn et al. 2021).

2 Three-Signal Model of the Alloimmune T Cell Response in the SLO

Alloimmune responses are initiated by activation of antigen-presenting cells (APCs) through innate immune recognition systems (Fig. 1). In the graft and surrounding tissues, dendritic cells of donor and host origin become activated and move to T cell areas of secondary lymphoid organs (SLO).

In the SLO, antigen-bearing dendritic cells engage alloantigen-reactive naive T cells and memory T cells (Fig. 1). While naive T cells are optimally triggered by dendritic cells in SLO (Lakkis et al. 2000; Zhou et al. 2003), previously stimulated or "antigen-experienced" memory cells may be activated by other cell types, such as graft endothelium (Biedermann and Pober 1998). This is an issue in clinical transplantation since human adults have large numbers of memory T cells activated previously by viral antigens that cross-react with alloantigens (Adams et al. 2003a) (heterologous memory (Adams et al. 2003b)). Some estimates indicate that many of the antigen-specific T cells reacting with donor antigens are memory T cells, not naïve T cells (Lombardi et al. 1990).

An antigen on the surface of dendritic cells that triggers T cells with cognate T cell receptors constitutes "signal 1," transduced through the CD3 complex. Dendritic cells provide costimulation, or "signal 2," delivered when CD80 and CD86 on the surface of dendritic cells engage CD28 on T cells. Memory T cells have less requirement for costimulation.

Signals 1 and 2 activate three signal-transduction pathways: the calcium–calcineurin pathway, the RAS–mitogen-activated protein (MAP) kinase pathway, and the nuclear factor-B pathway (Wang et al. 2004). These pathways activate transcription factors that trigger the expression of many new molecules, including interleukin-2, CD154, and CD25. Interleukin-2 and other cytokines (e.g., interleukin-15) activate the "target of rapamycin" pathway to provide "signal 3," the trigger for cell proliferation. Proliferation and differentiation lead to a large number of effector T cells.

B cells are activated when antigen engages their antigen receptors, usually in lymphoid follicles or in extrafollicular sites, such as red pulp of spleen (MacLennan et al. 2003), or possibly in the transplant (Sarwal et al. 2003), producing alloantibody against donor HLA antigens. However, follicular helper T cells are essential to the

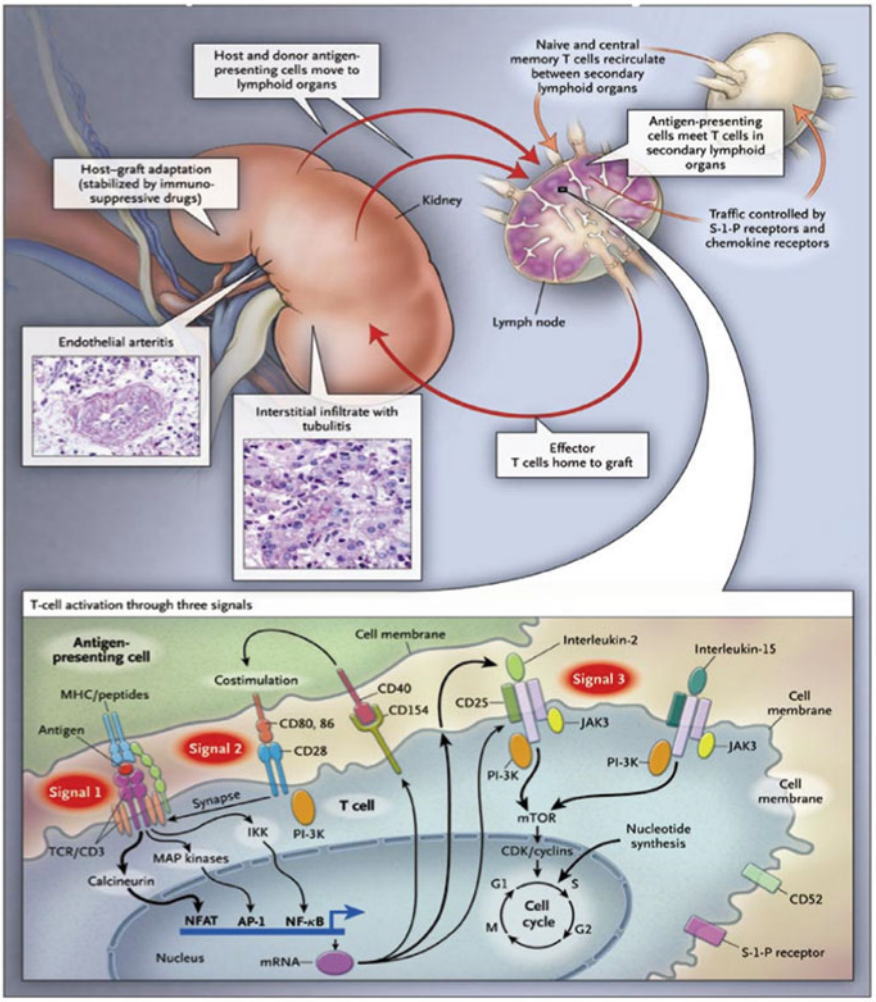

Fig. 1 Steps in T cell-mediated rejection. Antigen-presenting cells (APCs) of host or donor origin migrate to secondary lymphoid organs. APCs present donor antigen to naive and central memory T cells. These T cells ordinarily circulate between lymphoid tissues, regulated by chemokine and sphingosine-1-phosphate (S-1-P) receptors (Mandala et al. 2002). T cells are activated and undergo clonal expansion and differentiation to express effector functions. Antigen triggers T-cell receptors (TCRs) (signal 1) and synapse formation. CD80 (B7-1) and CD86 (B7-2) on the APC engage CD28 on the T cell to provide signal 2. These signals activate three signal-transduction pathways – the calcium–calcineurin pathway, the mitogen-activated protein (MAP) kinase pathway, and the protein kinase C–nuclear factor-B (NF-B) pathway – which activate transcription factors nuclear factor of activated T cells (NFAT), activating protein 1 (AP-1), and NF-B, respectively. The result is expression of CD154 (which further activates APCs), interleukin-2 receptor chain (CD25), and interleukin-2. Receptors for a number of cytokines (interleukin-2, 4, 7, 15, and 21) share the common chain, which binds Janus kinase 3 (JAK3). Interleukin-2 and interleukin-15 deliver growth signals (signal 3) through the phosphoinositide-3-kinase (PI-3 K) pathway and the molecular-target-of-rapamycin (mTOR) pathway, which initiates the cell cycle. Antigen-experienced T cells home to and infiltrate the graft and engage the parenchyma to create typical rejection lesions such as tubulitis and, in more advanced rejection, endothelial arteritis. However, if the rejection does not destroy the

generation of effective B cell transformation into mature plasma cells producing high-affinity IgG that can engage donor endothelium and produce ABMR.

Thus, within days the immune response generates the effector mechanisms that can damage the organ and mediate allograft rejection, effector T cells, and alloantibody. In naïve recipients the first rejection to appear is TCMR. New TCMR and ABMR responses can be initiated later, especially during periods of under-immunosuppression.

3 Effectors, Lesions, and Molecular Phenotype of Rejection

Rejection is defined as tissue injury produced by the effector mechanisms of the adaptive alloimmune response, leading to deterioration in organ function. Rejection has many dimensions: clinical, immunologic, molecular, and histologic.

There are two types of rejection: T cell-mediated rejection (TCMR) and antibody-mediated rejection (ABMR) (Fig. 2). TCMR, ABMR, and mixed rejection can be early or late, fulminant and rapid, or relatively indolent and slow. Increasingly new dimensions such as microarray or RNA sequencing analysis of genome-wide gene expression are being added.

T cells serve as the main effectors and regulators of the alloimmune response. Macrophages are possible effectors and aid in the removal of apoptotic cells. Theoretically, B cells and plasma cells could contribute to the production of alloantibodies within the graft but they are seen more often in TCMR and are not per se part of the criteria for ABMR. In ABMR, the high-affinity damaging IgG antibodies are probably made in SLO or the marrow.

3.1 T Cell-Mediated Rejection (TCMR)

Most rejection in clinical organ transplantation was previously TCMR, but effective ISDs have made TCMR later and less common, yet still important, e.g. in non-adherence. Cognate effector T cells that emerge from SLO infiltrate the graft and orchestrate an inflammatory response including recruitment of activated macrophages. Cognate effector T cells home to the graft by recognizing alloantigen on dendritic cell processes that emerge through the endothelium and guide the T cells through the capillary endothelium. In the interstitium they are then activated by dendritic cells to create the inflammatory environment that is the fundamental feature of TCMR (Halloran et al. 2016a), recruiting many other inflammatory cells:

Fig. 1 (continued) graft, adaptation occurs and is stabilized by immunosuppressive drugs. The photomicrographs of tubulitis and endothelial arteritis are taken from a mouse model in which these lesions are T cell-dependent but independent of perforin, granzymes, and antibody. IKK denotes inhibitor of nuclear factor-B kinase, CDK cyclin-dependent kinase, and MHC major histocompatibility complex. (Halloran, P. F. N Engl J Med 2004;351:2715–2729)

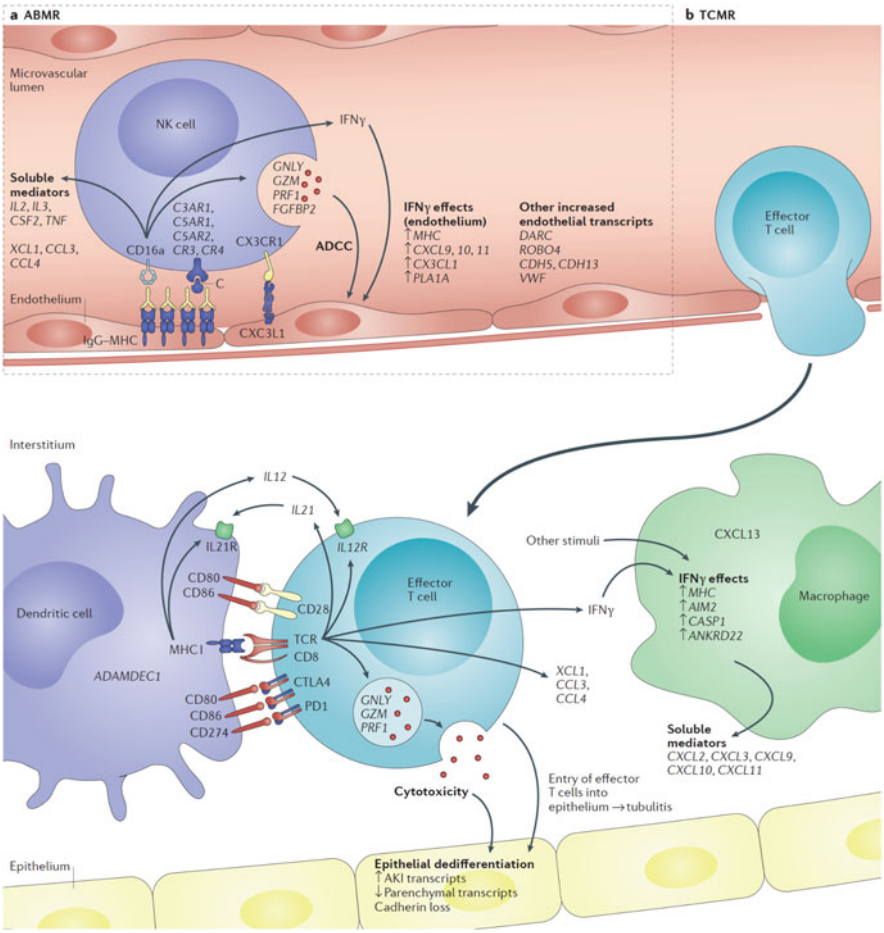

Fig. 2 (**a**) Tubulitis in T cell-mediated rejection (PAS, 40×) (**b**) Endothelialitis in T cell-mediated rejection (PAS, 40×)

non-cognate effector and memory T cells, macrophages, B cells, and plasma cells – the cellular infiltrate observed in TCMR biopsies. (The plasma cells seen in TCMR and damaged tissue generally are probably not fully mature and are not an important source of high-affinity alloantibody, which generally comes from mature bone marrow plasma cells.)

The diagnostic lesions of T cell-mediated rejection reflect mononuclear cells accumulating in the interstitium and in the cases of epithelial organs invading the epithelium, e.g. kidney tubules (tubulitis) and the intima of small arteries (arteritis) (Racusen et al. 1999). In the heart, the parenchyma manifests myocyte injury and necrosis. The molecular hallmark of all rejection is that the graft displays intense IFNG expression and IFNG-induced molecules such as CXCL9, CXCL10, and

CXCL11, accompanied in TCMR by many TCMR-selective transcripts expressed in effector T cells (e.g., IFNG), APCs, and macrophages (e.g., CXCL13 and ADAMDEC1), and checkpoint transcripts such as CTLA4. The parenchyma deteriorates with loss of the transcripts associated with normal function and expression of acute injury transcripts (Halloran et al. 2018; Venner et al. 2014; Venner et al. 2015; Loupy et al. 2017).

The recruitment of the other inflammatory cells into the graft across the microcirculation endothelium – diapedesis – is a result of the expression of chemokines and adhesion molecules by the endothelium of the graft. The steps are: rolling on selectins, engagement of chemokines, tight binding to adhesion molecules, then transendothelial migration. The endothelium of postcapillary venules serves as the entry point of recipient leukocytes from the bloodstream into the allograft. Endothelial cells are activated by proinflammatory cytokines and injury to express adhesion molecules and chemokines necessary for transendothelial migration. The recruitment of leukocytes is initiated by the release of chemokines by tubular cells, interstitial cells, endothelial cells, and infiltrating recipient cells within the allograft. T cells expressing the respective chemokine receptors extravasate through the endothelium and are guided by a chemokine gradient within the graft. The binding of chemokines to their receptors induces a conformational change in integrins, which are normally present on circulating leukocytes in an inactive state. Tight adhesion occurs when activated integrins bind their ligands on graft cells. The most common integrins present on lymphocytes are LFA-1 that binds ICAM-1 and -2, and VLA-4 that binds VCAM-1. Unfortunately, treating or preventing rejection by blocking adhesion has not been successful, likely due to redundancy among the multiple adhesion molecules and their ligands, and involvement of these mechanisms in many other types of inflammation.

An interesting but unexplained feature of rejection is that antigen-triggered effector T cells cross the donor endothelium without killing the endothelial cells. TCMR can smolder for days or weeks as an interstitial process, yet the graft remains viable. This could be related to T cell exhaustion.

3.1.1 Tissue Injury in TCMR

The main lesion for diagnosing kidney rejection in the Banff schema is tubulitis. E-cadherin on the basolateral membrane of the tubular epithelium of rejecting grafts may play a role in the development of tubulitis. Invasive lesions correlate with functional deterioration (Solez et al. 1993a, b) and may be relevant to the tubular atrophy that often follows rejection. TCMR can develop in mouse hosts lacking B cells and alloantibody (Jabs et al. 2003), and intense tubulitis is not a characteristic of human ABMR, although tubulitis is non-specific and can occur in ABMR and injury (Trpkov et al. 1996). In hearts, the corresponding lesion is myocyte injury and necrosis.

Alloimmune T cells may mediate parenchymal injury either through direct contact (cytotoxicity) or through contact-independent inflammatory mechanisms analogous to delayed-type hypersensitivity (DTH). Infiltrating effector T cells display many cytotoxic molecules: enzymes in their granules – perforin (Prf1),

granzymes A and B (GzmA/B), and granulysin (GNLY) – as well as Fas ligand on their membranes (Robertson et al. 1996; Einecke et al. 2005). CTL could engage or even synapse with epithelial cells via specialized molecules to damage individual epithelial cells via cytotoxic mechanisms. The enzymes from stored granules released into the cytosol of target cells could initiate a cascade of events that leads to apoptosis, and engagement of Fas on target cells by FasL can cause apoptotic death of the target cell. However, TCMR is not dependent on granule-associated CTL mechanisms: it can develop in allografts rejecting in hosts lacking Prf1 or granzyme A (GzmA) and granzyme B (GzmB) (Halloran et al. 2004) or Fas ligand.

It has been suggested that the integrin CD103, by binding its ligand E-cadherin on epithelial cells, may permit CD8 T cells to engage the renal epithelium (Hadley et al. 1999; Robertson et al. 2001) and mediate invasion into tubular cells. However, mice deficient in CD103 develop tubulitis and deterioration similar to wild-type hosts, indicating that CD103 is not critical for TCMR.

The independence of the epithelial deterioration from cytotoxic mechanisms suggests that the interstitial inflammatory cells such as macrophages and effector T cells produce epithelial dedifferentiation by synergy among inflammatory molecules such as IFNG and TNF in a general inflammatory process called "delayed-type hypersensitivity" (DTH). Parenchymal deterioration in DTH is mediated by contact-independent mechanisms. APCs and macrophages are activated by effector T cells participate in TCMR through DTH mechanisms (Bogman et al. 1989), but the injury remains antigen-specific (Rosenberg and Singer 1992). Mechanisms directly altering the epithelium could include the release of soluble effector T cell or macrophage products (cytokines, superfamily members, reactive oxygen species, nitric oxide, eicosanoids, and enzymes). Additional effects may operate by changing the extracellular matrix (e.g., synthesis of hyaluronic acid) or the microcirculation. Tubulitis in kidney transplants may be a relatively late change in the epithelium, reflecting loss of epithelial integrity that permits entry of lymphocytes, which would explain the lack of requirement for cytotoxic mechanisms and the occurrence of tubulitis in atrophic tubules independent of rejection. The conditions for tubulitis may simply be interstitial infiltration and compromised epithelial integrity, and the diagnostic value of tubulitis may be as an indicator of this loss of epithelial integrity.

While our current belief is that TCMR is at least in part an interstitial inflammatory process mediated by effector T cells with cytotoxic activity but via delayed-type hypersensitivity (DTH) mechanism, it is also possible that the T cells sometimes augment this via their cytotoxic mechanisms. The epithelium deteriorates and loses its ability to exclude inflammatory cells, permitting T cells to enter to create tubulitis. PRF1, GZMA, GZMB, GNLY, and FAS-FAS ligand may be supplementary but are not essential in this model.

The intensity of TCMR correlates with the expression of checkpoint molecules (see below), indicating that T cell mechanisms are never activated without activation of inhibitory processes. This is because of the vast power of T cell effector mechanisms to do harm. TCMR also becomes rare as the years go by, suggesting that the clonal T cells eventually become exhausted (Halloran et al. 2015).

TCMR is "treatable" but is still a serious event for an organ transplant because of its ability to directly damage the parenchyma (nephrons, myocytes, etc.). TCMR on current immunosuppressive protocols usually occurs in one of four situations:

1. In the first 3–12 months, often due to failure to sustain target ISD levels or ill-advised attempts to "minimize" ISDs below recommended levels;
2. Following ISD reduction associated with virus infections and other complications, where a TCMR-like process is common. Note that TCMR-like inflammation develops in some virus infections (e.g., polyomavirus infection in kidney transplants) and may be virus-immune or alloimmune (Halloran et al. 2021a);
3. In non-adherent patients;
4. During treatment of cancer with checkpoint inhibitors.

3.2 Antibody-Mediated Rejection

ABMR is now recognized as a major cause of loss of kidney and heart transplants (and possibly lung and liver transplants, although less is known about ABMR in these organs). ABMR is a major target for efforts to reduce transplant failure (Djamali et al. 2014; Einecke et al. 2009; Sellares et al. 2012).

Mechanism of ABMR. ABMR represents the effect of alloantibodies against donor antigens (donor-specific antibodies, DSA) binding to the graft microcirculation, leading to complement activation and margination of neutrophils, monocytes, and NK cells in the glomeruli and peritubular capillaries – glomerulitis and peritubular capillaritis – and endothelial injury (Halloran et al. 1990). The main antigenic targets of ABMR are MHC molecules, both class I and class II.

Alloantibodies against non-MHC proteins could potentially mediate ABMR but this has not been proven. Antibodies directed against self-proteins such as AT1R can be associated with graft injury (Dragun et al. 2005), but their role in ABMR is a matter of debate.

There is increasing recognition that ABMR often occurs in the absence of DSA recognized by current measurement platforms – see below.

3.3 Triggering of Host B Cell Clones with Cognate Receptors for Native Donor HLA Molecules

The B cell response to antigens generates germinal centers in lymph nodes as the B cells, helped by follicular helper T cells, undergo clonal expansion, class switching (IgM to IgG) and affinity maturation, leading to the production of plasma cells, which migrate to the bone marrow where they continue to produce antibody.

De novo IgG anti-HLA production requires triggering of host B cell clones with IgM receptors for donor HLA antigens to develop mature IgG-producing plasma cells producing anti-HLA IgG (Fig. 3). B cells in SLO engage the donor HLA

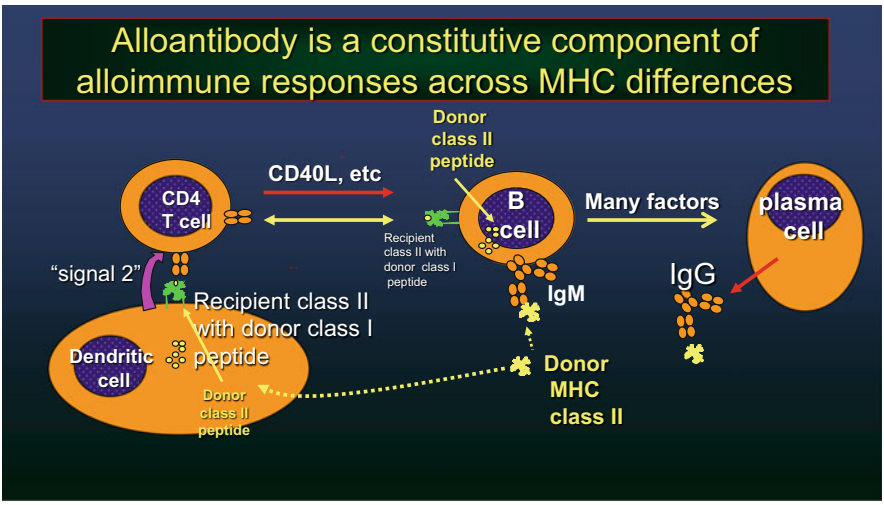

Fig. 3 Triggering of host B cell clones with antigen-specific receptors for donor HLA antigens to develop mature plasma cells producing anti-HLA IgG. B cells in SLO engage the donor HLA molecules and begin their triggering process, and express class II with donor HLA peptides. However, they require "help" from follicular helper T cells (TFH) that which recognize the donor HLA proteins as peptides in host class II molecules. The TFH are primed by host APC that have incorporated donor HLA antigen and expressed host class II with donor HLA peptides. The TFH then engage the B cells, which express the same host class II with donor HLA peptides, and provide help, permitting clonal expansion, affinity maturation, and generation of plasma cell precursors that eventually home to the bone marrow as mature plasma cells. Plasma cell maturation requires support from many molecules, including IL6

molecules and begin the triggering process, and express class II with donor HLA peptides. However, they require "help" from follicular helper T cells (TFH) that recognize the host class II molecules with donor HLA peptides. The TFH is primed by host APC that have non-specifically ingested donor HLA antigen and expressed host class II with donor HLA peptides. The TFH then engages B cells that express the same host class II with donor HLA peptides, and provides help, permitting clonal expansion, affinity maturation, and generation of plasma cell precursors that eventually home to the bone marrow as mature plasma cells. Plasma cell maturation requires support from many molecules, including IL6.

It is not known whether the long-term production of antibodies specific for donor antigens is maintained by long-lived plasma cells or by continuous generation of new memory B cells or both. Some late failing grafts near end-stage become "tertiary lymphoid organs" with organized lymphoid follicles (Colvin and Smith 2005). However, these late changes are agonal in near end-stage tissues and are never seen in early ABMR. High-affinity damaging IgG antibodies are probably produced by fully mature affinity matured bone marrow plasma cells.

3.4 Effector Mechanisms in ABMR

The potential effector functions of DSA against donor endothelium include direct effects (although this has not been demonstrated in vivo), complement activation, and recruitment of effector cells through engagement of Fc receptors (Colvin and Smith 2005; Lee et al. 2007). In general, IgG probably requires hexamer formation to activate complement (Lee et al. 2011) and possibly Fc receptors. Many IgG antibodies may be unable to form such hexamers, explaining why some apparent DSA may not produce injury.

Complement activation is often observed in ABMR but its actual role in most cases is questionable because blocking complement does not prevent the progression of ABMR (Bohmig et al. 2019). Complement-fixing DSA is more diagnostic for ABMR than non-complement fixing antibodies in kidney transplants (Loupy et al. 2013), but C4d deposition is not evident in many ABMR (Einecke et al. 2009; Sis et al. 2009; Haas 2011). Complement activation mediates injury by lysis or attracting inflammatory cells via chemoattractants C3a and C5a.

In severe cases of ABMR, glomerular capillary thrombosis can occur in ABMR, but thrombotic microangiopathy is very rare, and often due to other causes.

Leukocytes in the microcirculation in biopsies with ABMR (in kidneys, peritubular capillaritis or ptc-lesions and glomerulitis or g-lesions) are the main feature of ABMR, suggesting an effector role for these cells, but whether such cells are mediators of injury or are recruited by injury or both is difficult to establish. The strongest molecular associations point to NK cells. NK cells are a hallmark of ABMR, whether DSA-positive or DSA-negative. Activated NK cells produce IFNG and probably account for the IFNG effects in ABMR. NK cell transcripts such as KLRD1 are prominent of kidney and heart ABMR, and NK cells may be critical effectors of ABMR (Halloran et al. 2016a; Venner et al. 2015).

A possible mechanism of the microcirculation injury in ABMR is antibody-dependent cellular cytotoxicity (ADCC) through CD16a Fc gamma receptors on NK cells (Venner et al. 2015; Hidalgo et al. 2010). The principal Fc gamma receptor on human NK cells is CD16a (FcγRIIIa or FCGR3A), an activating receptor with signal-transducing mechanisms like the T cell receptor. Like effector T cell activation, CD16a triggers calcineurin and releases cytokines and cytotoxic molecules that induce injury and target cell apoptosis (Halloran et al. 2016a; Venner et al. 2015).

NK cells also have other activating and inhibitory receptors (Long et al. 2013), many with the ability to engage MHC class I, which may help them recognize "missing self" (Callemeyn et al. 2021). This raises the possibility of considering donor-recipient matching for NK receptors to avoid triggering NK recognition.

Studies of kidney and heart transplant biopsies provide strong support for the role of NK cells in ABMR syndromes (Parkes et al. 2017). These data support a model of ABMR inducing injury in the microcirculation endothelium, induced by donor-specific antibody or missing self mechanisms (e.g., CD16a activation of NK cells, triggering IFNG release and NK cell-mediated ADCC (Venner et al. 2015)).

Clinical presentations of ABMR. The dynamic range of ABMR is highly variable, from fulminant failure within hours (Patel and Terasaki 1969) to a relatively indolent course progressing over years (Sis et al. 2007) or even stable or burned out. ABMR is diagnosed by clinical (Halloran et al. 1990), immunologic (Terasaki 2003), and histologic criteria. The key kidney histology lesions in ABMR are microcirculation inflammation (peritubular capillaritis and/or glomerulitis lesions) and glomerular double contours (cg lesions). Hearts with ABMR have microcirculation inflammation but lack chronicity lesions for staging. ABMR can produce arteritis, like TCMR, although this is relatively uncommon. The Banff and ISHLT guidelines are empirically derived to achieve a reasonable trade-off between over- and underdiagnosis. Both recognize that DSA may not be demonstrable (Halloran et al. 2017).

New insights on the phenotypes of ABMR come from molecular assessment of ABMR phenotypes (Reeve et al. 2017; Venner et al. 2015). By molecular analysis, ABMR occupies a continuum of molecular space from early-stage to fully developed to late-stage (and even burned out) in the natural history of ABMR (Reeve et al. 2017). At least 25% of MMDx ABMR is DSA negative (Einecke et al. 2021).

3.5 Classification of ABMR

It is useful to characterize ABMR as

1. Hyperacute, mediated by very high levels of circulating DSA at transplantation;
2. Type 1 ABMR, mediated by re-emergence of a previously sensitized DSA due to memory, producing large amounts of DSA in the early post-transplant period;
3. Type 2 ABMR, mediated by the later appearance of ABMR independent of previous sensitization, often due to a documented de novo DSA. Of interest, type 2 has a poorer prognosis than type 1 (Aubert et al. 2017). Most ABMR now is type 2 with de novo DSA. Type 1 ABMR may do better because for unknown reasons the presensitized DSA response may eventually attenuate and disappear on immunosuppression, unlike most de novo DSA.

3.5.1 Hyperacute ABMR

This condition is prevented by crossmatching and is virtually never encountered unless a serious error is made in selecting donors. If recipients have been sensitized by previous transfusions, pregnancies, or transplants bearing donor MHC molecules, they may have high levels of pre-formed alloantibody against the donor. This can lead to disastrous hyperacute rejection, even on the operating table (Kissmeyer-Nielsen et al. 1966). Similar changes occur with incompatibility between donor and recipient at the ABO blood group locus if A- or B-antibodies are high-titer, analogous to incompatible blood transfusion. In these cases, the entire endothelium of the graft is injured, and the large vessels usually fail, leading to immediate complete failure of the graft.

The existence of pre-formed alloantibodies against HLA or AB antigens can be detected by crossmatching and ABO matching prior to transplantation to prevent

hyperacute rejection. Effective crossmatching effectively eliminated such cases, except for catastrophic failures of the safety checks.

3.5.2 Early Acute ABMR in Sensitized Patients (Type 1)

Early ABMR in the days post-transplant reflects an anamnestic burst of donor-specific HLA antibody, classically associated with the triad of decreased renal function, the presence of circulating DSA, and histological evidence of active antibody-mediated tissue injury (microvascular inflammation) (Trpkov et al. 1996), and often with deposition of complement component 4d (C4d) in peritubular capillaries (Feucht 2003). (ABO incompatibility can cause very early ABMR if the levels of antibody are high but is usually well tolerated after the initial period, despite C4d staining.) This ABMR phenotype can also emerge in the next few weeks. Type 1 kidney ABMR presents as early-stage molecular features with ptc- and g-lesions, and progresses to fully developed molecular features (glomerular double contours) over the next year.

3.5.3 ABMR Apparently Independent of Pre-Transplant Sensitization (Type 2)

Type 2 ABMR, by far the commonest type of ABMR, presents as early-stage ABMR (EABMR) in its molecular features and ptc- and g-lesions. Like type 1 ABMR, type 2 progresses to fully developed ABMR (FABMR) with histologic glomerular double contours, usually after at least 12 months. However, EABMR often escapes detection and presents as FABMR.

Type 1 EABMR usually is observed in high-risk transplants. Type 2 EABMR starts to become common to become common at the end of the first year post-transplant, and new cases continue to appear indefinitely, with molecular findings: NK transcripts and IFNG-inducible transcripts associated with histologic ABMR-related lesions peritubular capillaritis and glomerulitis.

The features of type 1 and type 2 are identical at the FABMR stage, include NK transcripts, IFNG-inducible transcripts, and certain endothelial transcripts such as ROBO4, as well as the triad of histologic ABMR-related lesions: peritubular capillaritis, glomerulitis, glomerular double contours). Double contours (duplication of the glomerular basement membrane or transplant glomerulopathy) are accompanied by lamination of the peritubular capillary basement membrane (Mauiyyedi et al. 2001; Regele et al. 2002; Vongwiwatana et al. 2004). These changes represent stages of progression of microcirculation changes after many months of ABMR (Lefaucheur et al. 2013; Cosio et al. 2008; Loupy et al. 2014). It would be useful to find such ABMR staging lesions in heart ABMR.

3.6 Late-Stage ABMR (LABMR)

FABMR often progresses to LABMR after several years, with atrophy-fibrosis and glomerular sclerosis. DSA may become negative, perhaps reflecting immune adaptations and long-term immunosuppression, or perhaps the natural history of

the antibody response. Moreover, new-onset EABMR becomes uncommon after 10 years, perhaps reflecting the adaptations in TFH.

3.7 Mixed Rejection

This phenotype is frequently seen in severe TCMR, often associated with non-adherence (Halloran et al. 2016b) and with intimal arteritis (v-lesions). A characteristic is a lack of afferent arteriolar hyalinosis due to inadequate exposure to calcineurin inhibitor ISDs (Einecke et al. 2017). A common presentation is severe TCMR followed by emergence of early-stage ABMR. With treatment of TCMR, and given the difficulty of treating ABMR, ABMR may then become the dominant long-term phenotype.

3.8 Sub-Threshold ABMR-Like Changes

We have recently found that mild ABMR-like changes exist in many biopsies that are currently diagnosed as no rejection, often but not always associated with DSA (Madill-Thomsen et al. 2021; Halloran et al. 2021b). At least in kidney transplants, the grafts with these changes are at risk of future deterioration.

3.9 DSA-Negative ABMR

ABMR molecular and histologic features can be found in kidneys and hearts in patients with no demonstrable DSA. In kidneys, the mean time of onset is somewhat earlier than DSA-positive ABMR, but the same genes are induced, e.g., NK transcripts and IFNG-induced transcripts although with moderately lower expression; the same histology microcirculation lesions are present; and both impair graft survival. Thus at least in kidneys, DSA-negative ABMR presents as an earlier and milder form of the same disease as DSA-positive ABMR.

The possible mechanisms operating in DSA-negative ABMR include:

1. Anti-HLA that is not detected by the usual platforms such as Luminex.
2. NK cell recognition of missing self HLA proteins such as HLAC.
3. Antibody against non HLA alloantigens.
4. Autoantibody.

3.10 Unsolved Issues in ABMR

It is clear that there are fundamental issues that need to be addressed in ABMR. What is the natural history of DSA and of ABMR? Can DSA and ABMR spontaneously disappear? What determines the pathogenicity of DSA? How can silent ABMR

phenotypes be detected in the clinic, and if they can, how should they be managed? What is the mechanism of DSA-negative ABMR, and if it is DSA that is not detectable by current tests, what is the target antigen, and how can we detect this antibody? Above all, there is a need for a safe and effective way of suppressing ABMR without putting patients at risk since current treatments are far from satisfactory.

4 Donor-Derived Cell-Free DNA (dd-cfDNA)

We do not have time for a detailed cover of dd-cfDNA; this subject has recently been reviewed (Kataria et al. 2021), and this is an active area for investigation as a blood screening test for rejection. ABMR and to a lesser extent TCMR and tissue injury release donor cfDNA, which has a short half-life and represents a potentially useful signal for monitoring the organ. The utility and cost-effectiveness of dd-cfDNA for monitoring organ transplant patients are under review (Puttarajappa et al. 2021).

5 Host-Graft Adaptation

Over many years, transplant patients on current ISD protocols develop reduced T cell responsiveness to donor antigens, although they still require immunosuppression. Clonal T cell responses to donor alloantigens, which are required both for generating effector T cells for TCMR and helper T cells for new DSA production to initiate ABMR, have constitutive controls that are activated from the first steps in the response. These controls are needed to avoid uncontrolled proliferation and to avoid destruction of host tissues if viruses cannot be cleared. Some are intrinsic to the cognate T cells clones, e.g., exhaustion. Others reflect those actions on these clones of regulatory cells such as Tregs. The term "host–graft adaptation" describes the decrease in both donor-specific responsiveness and the risk of rejection in the years after a successful transplantation maintained with immunosuppression (Starzl et al. 1963). Changes in the organ – a loss of donor dendritic cells ("passenger leukocytes") and resolution of injury – probably play little if any role. The crucial element is the change in the cognate clones: anergy or clonal exhaustion.

5.1 Immune Checkpoint Molecules

Exhaustion is a general characteristic of T cell responses in vivo when antigen persists (Schwartz 2003) and is mediated by immune checkpoints, which was the basis of the 2018 Nobel prize in medicine for J. P. Allison and T. Honjo. Immune checkpoint molecules represent surface molecules on T cells that engage ligands and act as brakes on the T cell system that are essential for induction of exhaustion and the maintenance of immune homeostasis. The suppressive functions of immune checkpoints usually depend on ligand-induced signaling. These receptors often use

mono-tyrosine signaling motifs, such as immunoreceptor tyrosine-based inhibitory motif (ITIM) and immunoreceptor tyrosine-based switch motif (ITSM), to deliver inhibitory signals. Inhibitory immunoreceptor-ligand combinations include PD1, CTLA4, and their ligands (He and Xu 2020).

CTLA4 is structurally related to CD28 and binds CD80/B7.1 and CD86/B7.2 with greater affinity and avidity than CD28, thus enabling it to outcompete CD28 for its ligands. CTLA4 transmits an inhibitory signal to T cells, thereby limiting T cell immune responses (Sansom 2000). PD-1 is expressed on antigen-activated T cells and upregulated in T cell exhaustion (Mumprecht et al. 2009). In the presence of its ligands (PD-L1/PD-L2) on the surface of APCs and peripheral tissue, PD-1 signaling results in decreased proliferation, IL-2 production, protein synthesis, and survival of T cells (Francisco et al. 2010), thereby suppressing T cell inflammatory activity. PD1 ligands are inducible by IFNG, helping them to control TCMR.

In addition to the CTLA4-B7 and PD1-PD1L mechanisms, other checkpoints could be relevant to transplantation, including BTLA, CD160, LAG3, TIGIT, CD244/2B4, and HAVCR2/TIM3. All of these genes show increased expression in TCMR.

As surface molecules, the activity of these checkpoints can be inhibited by blocking antibodies that prevent ligand-receptor engagement, and this forms the basis of successful anti-cancer therapy. Like organ allografts, cancer represents persistence of antigen and induces adaptations that limit effector T cell generation. The most successful immune checkpoint blockade therapy for cancers targets PD-1/PD-L1 and has been approved to treat a wide variety of cancers (Ribas and Wolchok 2018).

In transplant patients, immune checkpoint inhibition is a considerable risk for TCMR (Manohar et al. 2020; Abdel-Wahab et al. 2019). Although patients with long-term transplantation are less prone to acute rejection, there was no correlation between the rate and timing of checkpoint-inhibitor-induced allograft rejection and the time since transplantation in those patients treated with checkpoint inhibitors. Although spontaneous TCMR occurs only rarely beyond 10 years after transplantation, catastrophic TCMR can occur after checkpoint inhibition (Lipson et al. 2016). Transplant biopsies demonstrated an acute TCMR process in half of the patients who received checkpoint inhibitor therapy, even at 25 years after transplantation. ABMR is usually not induced by checkpoint inhibitors. These observations suggest that the PD-1 pathway (as well as other checkpoints) stabilizes the T cell system in transplant patients and contributes to long-term graft stability.

5.2 Regulatory T Cells

Regulatory T cells (Tregs) emerged as a mechanism in the control of autoimmunity (Sakaguchi et al. 2001; Kim et al. 2007; Lahl et al. 2007), and considerable interest has focused on their role in organ transplantation and their potential for cell-based therapy (Wood and Sakaguchi 2003; Fehervari and Sakaguchi 2005). Such studies often incorporate the transcript factor forkhead box P3 (FOXP3), a forkhead-winged

helix transcription factor important in the development and function of Tregs (Ziegler 2006; Walker et al. 2003; Yagi et al. 2004). Foxp3 knockout mice exhibit severe systemic autoimmune-like syndrome (Chikuma and Bluestone 2007; Sharma et al. 2007). Humans with mutations of FOXP3 manifest X-linked IPEX syndrome: immune dysregulation, polyendocrinopathy, and enteropathy (Wildin and Freitas 2005). Thus FOXP3 is important in cells that regulate self-tolerance.

In human organ transplantation, the significance of FOXP3+ cells remains unclear. In transplant biopsies for cause, FOXP3 mRNA expression is not a feature of pristine transplants but transplants with rejection, inflammation, and injury (Bunnag et al. 2008). FOXP3 expression in kidney tissue is a feature of renal inflammation, which is never beneficial compared to the absence of inflammation, but within such inflammation FOXP3 positive Tregs may help stabilize the inflamed site. In addition, FOXP3 positive Treg cells may be stabilizing T cell responses in SLO, preventing effector T cell generation. FOXP3 positive Tregs probably contribute to the control of all immune responses, including alloimmune responses, by analogy with their ability to suppress autoimmunity (Sakaguchi 2004). But the importance of Tregs in the events in individual patients has not been demonstrated.

Note that some researchers propose to inject regulatory cells as "drugs" to help immunosuppress transplant patients (Miller et al. 2004), but their short half-life makes this very challenging and no benefits have been shown.

5.3 Transplant Tolerance

Tolerance is a state of non-responsiveness to specific antigens induced by previous exposure to those antigens in an immunocompetent host. Transplant tolerance would allow organ transplantation without ISDs and risks of infection and cancer if it could be induced safely and last indefinitely. Unfortunately, there is no current strategy that has been proven to induce durable safe long-term transplant tolerance for HLA antigen mismatched organ transplants.

6 Late Slow Deterioration of Organ Transplants

Much of late kidney and heart loss occurs after late slow deterioration of graft function, characterized by characteristic histologic and molecular changes and loss of GFR, usually with proteinuria. (The term chronic rejection is not useful and should be avoided. If TCMR or ABMR are present use those terms.) Kidney histology shows parenchymal atrophy, interstitial fibrosis, fibrous intimal thickening of arteries, and hyalinosis of afferent arterioles. MMDx shows transcripts associated with atrophy-fibrosis: transcripts for plasma cells and B cells (Einecke et al. 2008) and mast cells (Mengel et al. 2009), AKI molecules (Einecke et al. 2010), and loss of normal parenchymal transcripts (Venner et al. 2016). These are the features of irreversible parenchymal loss, the final common pathway of many diseases (Risdon

and Sloper 1968), and reflect the cumulative burden of injuries, perhaps superimposed on advancing organ aging.

Some late slow graft deterioration is due to late uncontrolled antibody responses, but recurrent primary disease can produce similar results.

Many late losses remind us of the need for life-long immunosuppression and surveillance of renal transplant recipients, and the risks of graft loss if we "minimize" immunosuppression. The contribution of non-adherence to graft loss is considerable (Sellares et al. 2012), often presenting as TCMR but evolving to ABMR if the TCMR is treated. Understanding, preventing, and managing under-immunosuppression and non-adherence remains a major unsolved problem in organ transplantation.

The problem of parenchymal loss and deterioration of function must be seen in the context of the natural history of the organ with aging, beyond transplantation. Some parenchymal loss (atrophy-fibrosis) often manifests in the first year due to the effects of donation-implantation injury after the early injury response has resolved, and is not progressive (Venner et al. 2016). But progression often reflects some new injury process such as rejection, infection, or primary diseases, or in kidney CNI toxicity (although CNI toxicity has been over-estimated in the past by reliance on hyalinosis, which can be due to donor age, glomerular global sclerosis (Einecke et al. 2017), or hypertension (Trpkov et al. 1996; Schneeberger et al. 1999; Racusen et al. 2002; Halloran 2002; Halloran et al. 1999; Bonsib et al. 2000; Solez et al. 1998). Parenchymal atrophy-fibrosis is not believed to be inherently progressive if the stress is terminated, e.g. withdrawal of CNIs if the cause is CNI toxicity, but more information is needed about how the parenchymal elements "remember" previous injuries, and whether there is a point of no return where progression becomes inevitable with no further insults.

7 Effects of Injury

Late allograft failure as a composite phenotype reflects the total burden of injury, including pretransplant factors, aging, and post-transplant immune and nonimmune injuries, plus limitations on organ homeostasis. Nonimmune stresses, such as brain death-related organ injury and warm and cold ischemia, and the stresses of preservation and implantation, have a direct effect on parenchyma and the circulation, acting as a challenge to homeostatic mechanisms.

In renal transplant populations, the probability of late graft loss is determined by five major groups of risk factors:

1. Organ "quality" (age, size, quality, and previous disease stresses, such as hypertension, cardiovascular disease, and diabetes, donor age);
2. Brain death;
3. Preservation and implantation injury (cold preservation plus rewarming, reperfusion);

4. Alloimmune injury (rejection): in human population data this is represented by the degree of HLA mismatch, sensitization, immunosuppressive drugs, and rejection episodes;
5. New stresses in the recipient environment (infection, hypertension, recurrent disease, drug toxicity, and advancing aging).

7.1 Does Injury Evoke Rejection?

While nonimmune injury and rejection injury can be additive, the basic science behind the relationships between injury and rejection is incomplete, and cannot be modeled in rodents or young primates because these models lack donor aging. We previously postulated that tissue injury, by evoking inflammation (innate immunity activation), increases the probability of rejection (Halloran et al. 1997), but this remains unproven. Living donor kidneys have some advantage in graft survival compared to deceased donor kidneys despite extensive HLA mismatching because they lack the injury associated with brain death and cold storage (Terasaki et al. 1995), but they are still at risk of rejection. Two kidneys from one deceased donor show similar function at all times post-transplant (Gourishankar et al. 2003), but they are not paired for rejection, which is driven mainly by non-donor factors.

Injury evokes response-to-injury effects on the organ as complex as the immune response itself (Halloran et al. 2021c). Inflammation – macrophage infiltration – is the normal response to wounding and should not be considered undesirable. However, the effects of injury on the parenchyma itself are often overlooked because the study of inflammatory and adaptive immune response is a natural priority of transplantation scientists. Inflammation is our hammer, and all the world looks like a nail. But the transplant patient wants high-quality parenchyma, and the reduction of parenchymal stress – peri-transplant stress, surgical and cold stress, ischemia, drugs, and infections – should be a priority, as well as understanding how to help injured parenchyma recover from these wounding effects.

8 Summary

The course of an organ transplant reflects the previous history of the organ (e.g., age, hypertension), its burden of injuries and stresses in the peri-transplant and post-transplant period, its post-transplant experiences, its intrinsic limitations on repair, and homeostasis imposed by aging and previous injuries, and rejection. The pathologic changes of rejection can explain how rejection can be associated with permanent loss of the limiting elements in an organ transplant. This puts the course of an organ transplant into the same context as the general problem of repair and homeostasis of that organ in the original host. The most preventable stress is rejection and identifying and treating all uncontrolled alloimmune injury remains the key to long-term graft stability. But in the long-term, the focus must include parenchymal health and homeostasis, and promotion of recovery from injury without atrophy-fibrosis.

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A Comprehensive Review of Calcineurin Inhibitors Used for Immunosuppression in Cardiac Transplantation

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Abstract

Calcineurin inhibitors (CNIs) have been the foundation of immunosuppression in solid organ transplantation since the 1980s. Cyclosporine A (CSA), the first in class, was identified as the metabolite of the soil fungus *Tolypocladium inflatum Gams* as part of a larger program of screening for naturally occurring fungal metabolites with biologic activity in the 1970s. Significant immunosuppressive effects were discovered and consequently CSA was trialed as an immunosuppressant in renal transplantation. This initial success led to its widespread study and adoption in solid organ transplantation. This novel agent yielded significant improvements in both 1 year and longer-term allograft and patient survival. Subsequently, a similar and more potent CNI, tacrolimus was developed. Today, it is the principal CNI used for prevention of allograft rejection. Like all other immunosuppressives, the benefits of CNIs are counterbalanced by side effects and complications resulting from drug toxicity. This chapter comprehensively reviews the clinical use of CNIs in cardiac transplantation.

Keywords

Calcineurin inhibitors · Complications of immunosuppression · Heart transplantation · Immunosuppression

1 History of Calcineurin Inhibitors

The introduction of cyclosporine as an approved medication to prevent transplant allograft rejection in 1980s, followed several years later by tacrolimus resulted in a new era of greater survival solid organ transplants with calcineurin inhibition. One-year heart transplant patient survival increased from around 60% to the mid 80% (Cheung and Menkis 1998; Cheng 2013). Both medications originate from the microbiome. Cyclosporine, the prototype, was a peptide isolated from the filamentous fungus *Tolypocladium inflatum* in Norway in the 1970s (Borel et al. 1994). Its immunosuppressant properties were discovered by J.F. Borel in 1976 and the FDA approved it for use for immunosuppression in solid organ transplantation in 1983. In 1987, tacrolimus was isolated as a macrolide antibiotic with immunosuppressive properties from the soil bacterium *Streptomyces tsukubaensis* by Tohru Kino in Japan (Hatanaka et al. 1988; Kino et al. 1987). It was approved for solid organ transplantation by the FDA in 1994. Since the late 1980s calcineurin based immunosuppression has been the standard for solid organ transplantation.

2 Mechanisms of Action

Despite differing microbiotic origins, cyclosporine and tacrolimus share similar mechanisms of action as calcineurin inhibitors. They both bind to immunophilins. Cyclosporine binds cyclophilins and tacrolimus (also known as FK-506) binds FK-binding proteins in the cytoplasm of cells (Matsuda and Koyasu 2000; Dumont et al. 1992). These immunophilins are present in almost all cells of the body, sparing the progenitor cells of the bone marrow, giving the medications a wide variety therapeutic and toxic potential. Once bound to their respective immunophilins, these complexes competitively bind to and inhibit the phosphatase calcineurin. Calcineurin inhibition results in reduced activity of the transcription factors, most significantly the nuclear factor of activated T cells (NF-AT), that are needed for cytokine gene transcription activation (Wuederrecht et al. 1993). Importantly, the transcription of interleukin-2 (IL-2) is impaired by calcineurin inhibition, resulting in suppression of both cell-mediated and antibody-mediated immune responses. Aside from the drug class nomenclature associated calcineurin inhibition, CNIs are also involved in the inhibition of the mitogen-activated protein kinase (MAPK) pathway responsible for T cell-mediated production of the proteins JNK and p38 (Matsuda and Koyasu 2003). JNK and p38 upregulate the transcription of IL-2 in the nucleus and thus IL-2 is inhibited by yet another different pathway (Clerk et al. 1999). It is also known that the cytokine TGF-B1, an inhibitor of IL-2 dependent T cell proliferation is increased in both cyclosporine and tacrolimus administration (Das and Levine 2008; Ahuja et al. 1995; Kwiek et al. 2008).

3 Medication Forms and Routes of Administration

There are several modes of administration available to choose from for calcineurin inhibition in solid organ transplantation that can be tailored to patient needs and preferences. Cyclosporine exists in oral, intravenous, and ophthalmic formulations. Tacrolimus exists in oral, intravenous, and topical formulations. There are both brand name and generic forms available for use. The ophthalmic formulation of cyclosporine and the topical formulation of tacrolimus are not for use in solid organ transplantation.

The oral formulation of cyclosporine is available in a nonmodified form that depends on bile for absorption (Sandimmune®) and also more commonly used microemulsion formulations that do not rely on bile salts for absorption and demonstrate increased area under the curve bioavailability (GENGRAF®, Neoral®). Both nonmodified and modified forms are available in capsule and liquid form. The oral formulation of tacrolimus is available in both immediate release and extended release formulations designed for once a day administration. While there are studies demonstrating the safety and efficacy of extending release tacrolimus (Astagraf XL® and Envarsus XR®) regimens in cardiac transplantation, FDA approval has not yet been granted for this indication (González-Vílchez et al. 2018). Tacrolimus also has a sublingual formulation that can be used when the enteric route needs to be

bypassed in the cases of both poor absorption and rapid metabolism (Pennington and Park 2015). In cases when an oral route cannot be used, the unmodified version of cyclosporine can be given as a continuous intravenous infusion. Tacrolimus also has a preparation for parenteral administration. It is important to recognize that when switching between routes of administration and formulations, dose conversions are generally not one to one. A table of the typical dose ranges used in cardiac transplantation is listed below.

Standard cardiac transplant calcineurin inhibitor dosing regimens

	Brand name	Oral capsule and liquid dosing	Sublingual dosing	Intravenous dosing
Cyclosporine				
Nonmodified, oil-based formulation	Sandimmune® (generics available)	4–8 mg/kg daily in two doses 12 h apart	N/A	1–2 mg/kg daily in two 2–6-h infusions or continuous infusion
Modified emulsion	Neoral® Gengraf® (generics available)	4–10 mg/kg daily in two doses 12 h apart	N/A	N/A
Tacrolimus				
Short-acting	Prograf® (generics available)	0.05–0.1 mg/kg daily in two doses 12 h apart	0.025–0.5 mg/kg daily in two doses 12 h apart	0.01–0.02 mg/kg daily in two doses or continuous infusion
Extended-release	Astagraf XL® Envarsus XR®	N/A	N/A	N/A

N/A indicates that the formulation is either not available or not applicable to cardiac transplantation

4 Pharmacodynamics and Therapeutic Drug Monitoring

While initial dosing of CNIs may be estimated based upon patient weight, these drugs are all dosed to maintain specified therapeutic drug levels. The various CNI formulations have different pharmacokinetic parameters and are not interchangeable. The doses of both cyclosporine and tacrolimus should be measured using a 12-h trough or a 24-h trough in the case of the extended release preparation of tacrolimus. This trough is noted as the concentration at time 0 or C0. There is a consensus that monitoring cyclosporine levels 2 h after administration (C2) can measure levels that better correlate with graft outcomes (Levy et al. 2002). However, the logistics of this in the outpatient setting makes this method less feasible. In general levels of both cyclosporine and tacrolimus are maintained highest immediately post-transplant and then decreased over time. The goal is to maintain the lowest levels possible to minimize side effects while preventing rejection. Target drug levels are often individualized for patients to account for varying toxicities, renal

dysfunction, rejection history, malignancies, and current infection status or risk of infections. In general there is a target cyclosporine trough of 275–375 ng/mL during the first six postoperative weeks, 200–350 ng/mL for weeks 6–12, and 150–300 ng/mL for months 3–6. Then a long-term maintenance goal of 150-250 ng/mL is targeted (Costanzo et al). For tacrolimus, the goal trough levels are typically 10–15 ng/mL in the first 3 months, with reduced target levels of 8–12 ng/mL in months 3–6, followed by a long-term maintenance goal of 5–10 ng/mL after 6 months (Costanzo et al. 2010).

5 Interpersonal and Intrapersonal Variations in Drug Metabolism

Individual patient differences exist in the amount of active medication per dosage and are accounted for by various factors. Differences in weight and body surface area can cause certain patients to require different doses. The generally low bioavailability of both cyclosporine and tacrolimus is related to poor gut absorption, degradation by enzymes in the GI tract, and first pass hepatic metabolism. Thus, malabsorptive conditions can prevent absorption which can affect levels of both drugs. In addition, there are genetic polymorphisms with some association to race that cause differences in the metabolism of calcineurin inhibitors. The amount of active medication after single dose has been noted to be less in Hispanic and African American subjects as compared to Caucasian subjects (Mancinelli et al. 2001). This variation has been traced to genetic polymorphisms in the CYP3A5 gene (Oetting et al. 2016). There are two alleles inherited from each parent that encode for each CYP450 enzyme. In addition, spontaneous mutations can occur. There are three different possibilities for inherited metabolism. If two wild type normal alleles are inherited, the individual will have a normal metabolism of the CNIs. If a wild type and a variant allele are inherited, the individual will have increased metabolism and will likely require a higher dosage of CNI to achieve therapeutic levels. If there are two variant alleles inherited, the individual may be an ultra-rapid metabolizer requiring even higher dosages of CNI or demonstrating an inability to maintain therapeutic CNI levels. These variations in metabolism can result in difficulties maintaining therapeutic CNI levels and make patient management challenging.

Some commonly available foods also affect levels of calcineurin inhibitors. For example, grapefruit contains furanocoumarins that are strong inhibitors of the cytochrome P-450 3A enzyme leading to decreased metabolism and increased and potentially toxic concentrations of cyclosporine and tacrolimus (Guo et al. 2000). Cyclosporine interacts with bile salts and thus a lipid rich meal can alter levels of the medication (Guan et al. 2011). As mentioned previously, both CNIs require absorption in the GI tract and thus medication preparations that coat the GI tract and prevention absorption can alter drug levels (Steeves et al. 1991). Another cause of intrapersonal variations in CNI metabolism are other medications which may be accelerators or inhibitors via interactions with the hepatic cytochrome 3A4 pathway.

Cyclosporine and tacrolimus also bind to the transporter, P-glycoprotein, making them susceptible to drug interaction from this pathway (Christians et al. 2005).

The effects of maternal ingestion of calcineurin inhibitors on the developing fetus and neonate also deserve consideration as women of childbearing age may become pregnant while taking calcineurin inhibitors that are needed for maintenance of a solid organ transplant. Due to the presence of the near ubiquitous immunophilins, both cyclosporine and tacrolimus cross the placenta and are transmitted in breast milk. Although long-term effects are not known, studies do show tolerability in gestation and a breast feeding (French et al. 2003).

6 Commonly Used Medications and Interactions with CNIs

The general management of post heart transplantation patients involves treating infections and utilizing infection prophylaxis. In addition, clinicians need to treat the cardiometabolic conditions associated with transplant and transplant immunosuppression. Below is a table of the effect on CNI levels in medications of drug classes that are commonly considered for use in transplant patients. In the treatment of conditions not included here, it is important in general to know if a new medication that is being introduced is an inducer or inhibitor of the cytochromeP450 3A4 system (CYP3A4) or the P-glycoprotein (P-gp) systems. In addition to the table below, it is important to note instances in which cyclosporine and tacrolimus levels may not be specifically affected but the newly introduced medications are more toxic or less effective as in the case of statins and diabetic medications.

Agents affecting CNI levels in medication classes commonly used post cardiac transplantation

Medication class	Mechanism	Cyclosporine levels	Tacrolimus levels
Antifungals			
Azoles Caspofungin	CYP3A4 and P-gp inhibition Unknown	↑ ↑	↑ ↑
Antibiotics			
Nafcillin Macrolides Metronidazole	CYP3A4 competition CYP3A4 inhibition CYP3A4 inhibition	↓ ↑ ↑	↓ ↑ ↑
Antihypertensives			
Diltiazem, verapamil	CYP3A4 inhibition	↑	↑

↑ indicates and increase in the CNI level and ↓indicates a decrease in levels

7 Side Effects and Complications of Calcineurin Inhibitor Use

The side effects and complications relate to the fact that the calcineurin inhibitors bind to cytoplasmic proteins that are present in most cells, excluding the bone marrow. While patients are spared the myelosuppressive effects seen in azathioprine, multisystem effects can be seen and need close monitoring. Infectious and malignancy complications throughout the body are unintended consequences of alternations of the body's immune defenses and should be screened for with use of all immunosuppressants, including calcineurin inhibitors. For the purposes of this book chapter and in clinical practice it is useful to categorize side effects by body systems. A review of systems can capture the side effects of these medications and concerns picked up during a review of symptoms can be addressed to potentially improve quality of life in transplanted patients.

8 Renal

Renal dysfunction is a particularly concerning complication of calcineurin inhibitor therapy. There is a risk of acute nephrotoxicity during initiation of CNIs in the postoperative period. This acute toxicity tends to be reversible. However, the chronic irreversible kidney disease that can develop as a result of long-term CNI use is considered a major downside. The negative effect on renal function is multifactorial and known to be related to damage to the renal arteries and arterioles, glomerulosclerosis, tubular atrophy, and interstitial fibrosis (Puschett et al. 1990). Generally, the clinical toxicity is based on a clinical assessment. However, a renal biopsy is the gold standard test to diagnose calcineurin-inhibitor-induced nephrotoxicity. Acute toxicity is characterized by necrosis and early hyalinosis of smooth muscle cells in the afferent arterioles, and isometric vacuolization of the proximal straight tubules. In chronic toxicity, medial smooth muscle cells in afferent arterioles are replaced by beaded hyaline deposits that bulge into the adventitia. There is also interstitial fibrosis and tubular atrophy (Liptak and Ivanyi 2006).

9 Cardiovascular

CNIs alter the vascular system via several different pathways. One that is most easily picked up clinically is the development of hypertension. CNIs induce hypertension by increasing sympathetic nerve activity, augmenting vascular tone, and altering kidney sodium transport (Hoorn et al. 2012). Long-term survival of the allograft places patients at risk for long-term consequences to the vasculature, notably cardiac allograft vasculopathy. Cardiac allograft vasculopathy differs from nontransplant related coronary disease in that it tends to be caused by accelerated intimal hyperplasia resulting in diffuse disease rather than focal stenosis or plaque rupture events. It is often a pernicious condition, especially in heart transplant patients who have denervation that prevents the sensation of chest pain. However, it is associated with

significant mortality. A review of the UNOS database found that around 6% of patients with CAV experience sudden cardiac death (Vakil et al. 2014). CNIs are not associated with the progression of cardiac allograft vasculopathy but they do not prevent progression like mTor inhibitors, and this may factor into decision making regarding their use.

10 Neurologic

Some general neurological toxicities associated with CNI use involve mental status changes, seizures, headaches, paresthesias, and most commonly tremor (Coe et al. 2020). Neuropathy is more frequent in a CNI immunosuppressive regimen as compared to a CNI free regimen and patient symptoms improve with withdrawal of CNI. This was found to be related to increases in nerve excitability parameters, suggestive of changes in nerve membrane depolarization (Arnold et al. 2013). A rare but important complication to be aware of is reversible posterior leukoencephalopathy syndrome (RPLS). It is also known as posterior reversible encephalopathy syndrome (PRES). This is a diagnosis that requires both clinical and radiographic findings. Clinical symptoms are hypertension in combination with headache, altered mental status, seizures, and visual disturbances. Neuroimaging will show posterior-predominant vasogenic edema best captured by MRI because of the posterior location. RPLS can occur at any time. It is most likely to occur shortly after the initiation of CNI and is associated with supratherapeutic levels (Anghel et al. 2013). Risk factors for the development of calcineurin inhibitors-related neurotoxicity are conditions that affect myelin or alter the blood brain barrier such as the use of methylprednisolone, infections, arterial hypertension, fluid overload, and hypocholesterolemia (Senzolo et al. 2009). Screening for neurologic complications is an important part of post-transplant management and special consideration should be given to transplant recipients with preexisting neurologic conditions.

11 Endocrine and Metabolic

The CNI carries a risk for the development of diabetes mellitus which is more pronounced in patients that are taking tacrolimus in comparison with cyclosporine (Heisel et al. 2004). The new onset diabetes after transplant (NOAT) is multifactorial in origin. It is a result of both reduced insulin sensitivity and reduced insulin secretion by pancreatic cells (Chakkera and Mandarino 2013). There are also alterations in bone metabolism turnover seen with CNI, especially with cyclosporine that can contribute to the development of osteoporosis (Thiébaud et al. 1996). These effects are attenuated by concomitant steroid use. Alterations in lipid metabolism occur with calcineurin use that contributes to cardiovascular disease mentioned prior. CNIs, most commonly cyclosporine, can interfere with renal tubular excretion of uric acid and cause gout in some patients (Brigham et al. 2020).

12 Gastrointestinal

Some common side effects of the CNIs are nonspecific nausea, vomiting, diarrhea. At high levels, both cyclosporine and tacrolimus can cause cholestasis (Oto et al. 2010). However, tacrolimus has better GI handling producing superior outcomes in graft function in liver transplantation (McAlister et al. 2006). The gastrointestinal system may also be a site for post-transplant lymphoproliferative disorder that is related to increase immunosuppresion (Stojanova et al. 2011).

13 Integumentary

Skin cancers are the most common type of malignancy in transplant recipients on immunosuppression with calcineurin inhibitors with squamous cell carcinoma being the most common type (Euvrard et al. 2003). It is believed that cancer is triggered by the production of TGF-B1 associated with CNI use and IL-2 levels are lower in patients with post-transplant skin cancers (Imko-Walczuk et al. 2016). Patients should be advised to avoid sun exposure, wear sunscreen, perform self-administered skin checks and have regular expert dermatologic evaluations. Gingival hyperplasia, especially in the presence of nifedipine, can occur with cyclosporine use (Dongari et al. 1993). Hair tends to be affected differently depending on the calcineurin inhibitor chosen. Hirsutism is more common with cyclosporine while alopecia is a more common finding associated with the use of tacrolimus (Yamamoto and Kato 1994).

14 Calcineurin Minimization

A goal in post-transplant therapy is to use the lowest effective dose to preserve graft function. This is the role of frequent laboratory monitoring of immunosuppressant drug levels to ensure patients are in a therapeutic window while avoiding supratherapeutic values more associated with toxicity. Aside from monitoring to ensure that levels are in a therapeutic range and using the lowest effective dose, there are strategies of CNI minimization. An early transplant strategy for CNI minimization is the delay of the initiation of CNI immediately post-transplant. This is facilitated using immunosuppression induction agents. Basiliximab and thymoglobulin are the most commonly used induction agents for this purpose and are associated with less renal dysfunction in the immediate postoperative period (Rosenberg et al. 2005; Cantarovich et al. 2004; Ekberg et al. 2009). Another strategy for CNI minimization involves the use of other immunosuppressive agents with differing mechanisms of action that allow for reduced CNI dosing and in some cases complete CNI withdrawal. Steroids, mycophenolate mofetil, and notably mTOR inhibitors have been utilized for this strategy.

15 Conclusion

The advances in heart transplantation along with concomitant immunosuppression, of which calcineurin inhibition has a major role, have provided a mechanism of increased survival in people with advanced heart failure. Special attention to CNI related drug interactions and CNI toxicity both acutely and chronically is important to assure successful management of the organ transplant recipient. The role of clinicians taking care of post-transplant patients on calcineurin inhibitors involves close drug monitoring, assessment for the known possible multisystemic effects, and adjustments as needed to obtain an appropriate balance between mitigating side effects and obtaining graft saving immunosuppression.

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Antiproliferatives and Transplantation

Robert Donovan, Howard Eisen, and Omaima Ali

Contents

Abstract

Antiproliferative agents include Mycophenolic acid and Azathioprine (which is less commonly used unless in certain conditions). They were initially identified for use in autoimmune and cancer research due to their role in disruption of cellular replication. They have now become the cornerstone of antirejection maintenance therapy in solid organ transplant. In this chapter we will describe the major times that lead to discovery, mechanisms of action, side effects, use during pregnancy and the major clinical trials.

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Antiproliferatives · Azathioprine · Clinical trials · Discovery · Mechanism of action · Mycophenolic acid · Side effects

1 Discovery

The antiproliferative medications azathioprine and mycophenolic acid (MPA) were both first identified as part of research into autoimmune diseases and cancer signaling pathways in humans. Before the structure of DNA was elucidated, a research team led by Gertrude B. Elion and George H. Hitchings studied an extensive number of purine analogs in the hope that they could discover a compound that could interrupt cellular replication (Elion 1989). In 1951, this team first synthesized 6-mercaptopurine (6-MP) which was initially approved for use in the treatment of childhood leukemias (Elion et al. 1960).

Meanwhile, after several advances were made in understanding the immunologic basis of organ transplantation and rejection, a separate team led by Sir Roy Calne began to use 6-MP in experimental attempts at human kidney and heart transplants (Schwartz et al. 1958). Elion and Hitching later synthesized a metabolic derivative of 6-MP in 1957 named *BW 57-322*, later termed azathioprine (Elion 1989). In collaboration with Elion and Hitching, Calne began to use azathioprine in place of 6-MP due to its more favorable side-effect profile. In 1954, the first successful living kidney transplant between identical twins was performed in Boston by Joseph Murray and his team. This work contributed to receiving the Nobel Prize for Medicine later on. In 1962, Calne and his team utilized an immunosuppressive regimen of azathioprine and glucocorticoids to perform the first successful long-term kidney transplantations from non-related donors (Murray et al. 1963).

Mycophenolic acid (MPA) was first synthesized in the late nineteenth century by an Italian medical scientist Bartolomeo Gosio. Using samples collected from spoiled corn, he discovered the fungal species *Penicillium brevicompactum* which had considerable antibacterial activity. In 1896, he isolated the crystallized form of the compound which gave the fungus its antibacterial properties (Zhang and Demain 2005). His discovery was initially forgotten until two American scientists, C.L. Alsberg and O.M. Black, later synthesized the same compound in 1912, giving it the name mycophenolic acid (Regueira et al. 2011).

MPA was initially used for its antibacterial and antiviral effects, though its adverse side-effect profile led to its near abandonment in clinical use. This changed in the 1980s due to the research of South African geneticist Anthony Allison and his wife Elsie Eugui. Allison discovered the metabolic pathway of de novo guanine nucleotide biosynthesis, particularly the enzyme Inosine-5'-monophosphate dehydrogenase (IMPDH), which is partly responsible for immune rejection in organ transplantation.

In their search for a molecule that could block this pathway, the Allisons experimented with the neglected antibacterial agent MPA, which they found to have significant immunosuppressive activity in mice and strong inhibition of mitogenic stimulation of human lymphocytes (Bentley 2000). After working to synthesize variants with less toxicity and increased immunosuppressive effect, they went on to demonstrate that MPA was useful in animal models of organ transplantation which was later extrapolated to humans in clinical trials (Bechstein et al. 1992; Taylor et al. 1994). MPA was then approved for use in kidney transplantation by the FDA in May of 1995 under the brand name CellCept®.

2 Mechanism of Action

2.1 Azathioprine

Although azathioprine is no longer used routinely in solid organ transplantation protocols, it was one of the first immunosuppressive agents in the field. Currently use is limited to stable patients already on therapy, those intolerant to mycophenolate acid due to GI side effects, or female transplant recipients considering pregnancy.

Azathioprine is a purine analog prodrug, which is rapidly hydrolyzed to 6-mercaptopurine (6-MP) after administration. 6-MP is later converted by hypoxanthine guanine phosphoribosyl transferase (HGPRT) to various metabolites including active 6-thioguanine (6-thioGTP), which becomes incorporated into actively replicating DNA preventing the de novo pathway of purine synthesis (Maltzman and Koretzky 2003). More specifically, 6-thioGTP has been shown to prevent DNA synthesis in actively replicating T cells. Thiopurine S-methyltransferase (TPMT) methylates 6-MP into the inactive form 6-methylmercaptopurine (6-MMP) (Fig. 1).

Recently, the same compound has been shown to inhibit CD28, a co-stimulatory mediator essential for the signaling pathway required for T cell activation (Aarbakke et al. 1997). Related metabolites of azathioprine have also demonstrated inhibition of the enzyme Rac-1, which sets in motion a series of pathways that culminate with mitochondrial-driven T cell apoptosis (Poppe et al. 2006). In TPMT enzyme deficient patients, toxic levels of 6-thioGTP can accumulate leading to life-threatening myelosuppression. Hence TMPT genotyping is recommended prior to initiation of azathioprine (Relling et al. 2013).

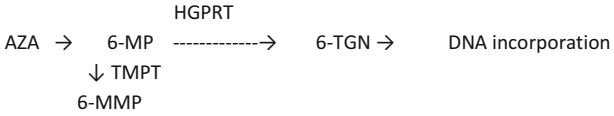

Fig. 1 Pharmacokinetics of MMF. *AZA* Azathioprine, *6MP* 6-Mercaptopurine, *HGPRT* Hypoxanthine guanine phosphoribosyltransferase, *6TGN* 6-Thioguanine nucleotides, *TMPT* Thiopurine S-methyltransferase, *6-MMP* 6-methylmercaptopurine

2.2 Mycophenolic Acid

MPA is a reversible inhibitor of Inosine monophosphate dehydrogenase IMPDH, a crucial enzyme in the de novo biosynthesis of guanine nucleotides. Mycophenolate mofetil (MMF, brand name CellCept®) undergoes rapid hydrolysis to the active form MPA after administration (Ransom 1995). The mean half-life of MPA in systemic circulation is approximately 17 h. MPA is mainly metabolized by the liver, undergoes glucuronidation to a pharmacologically inactive 7-O-glucuronide metabolite (MPAG) (major metabolite) and active metabolite MPA-acyl-glucuronide (AcMPAG) which is responsible for the GI toxic effects (Jeong and Kaplan 2007).

It undergoes enterohepatic circulation which contributes to approximately 35% of the MPA area under the curve (AUC). This leads to a secondary plasma peak after 6–12 h from administration. Cyclosporin inhibits this enterohepatic pathway for MPA lowering overall MPA plasma levels. MPA is eventually excreted through the kidneys (Jeong and Kaplan 2007) (Fig. 2).

While most cells in the human body can recover guanine nucleotides through salvage pathways, proliferating lymphocytes are entirely dependent upon the IMDPH pathway for purine synthesis and thus DNA replication (Ji et al. 2006). This partial selectivity for lymphocyte proliferation accounts for MPA's superior side-effect profile and efficacy when compared to azathioprine. MPA has been shown in in vivo experiments to block both T and B cell proliferation, and to down-regulate the expression of adhesion molecules on lymphocytes (Ensley et al. 1993).

3 Optimal Dose of Mycophenolic Acid

Clinical trials were conducted to assess optimal dosage, when comparing 2 g/day versus 3 g/day there was no additional benefit shown and a trend to more side effects with the higher dose, rendering 2 g/day as the standard of care. (Placebo-controlled study of mycophenolate mofetil combined with cyclosporin and corticosteroids for prevention of acute rejection. European Mycophenolate Mofetil Cooperative Study Group 1995).

4 Enteric-Coated Mycophenolic Acid

In an effort to reduce the gastrointestinal side effects of MMF, enteric-coated mycophenolate sodium (EC-MPS) was developed. This formulation allows for delayed release of MPA in the small intestine. Clinical trials showed similar safety profiles and efficacy including similar rates of biopsy proven rejection, graft loss,

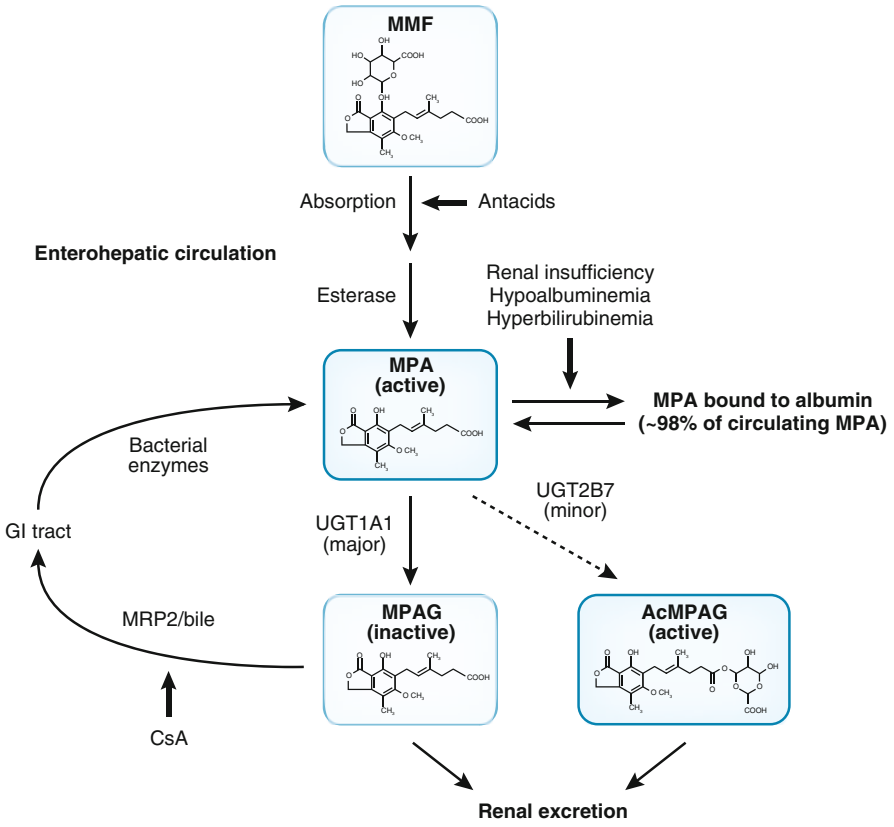

Fig. 2 Pharmacokinetics of MMF. *MMF* Mycophenolate mofetil, *MPA* Mycophenolic acid, *MPAG* inactive 7-O-glucuronide metabolite, *AcMPAG* active metabolite MPA-acyl-glucuronide. From "Therapeutic Monitoring of Mycophenolate Mofetil" by Hyunyoung Jeong and Bruce Kaplan. CJASN January 2007, 2 (1) 184–191. Reprinted with permission

and death. However, gastrointestinal adverse events were also found to be similar (Salvadori et al. 2004).

5 Target Dose Monitoring of MMF

Clinical trials showed a strong association of MPA concentration with incidence of rejection and individual variation of MPA AUC and pre-dose concentration, which led some to advocate for target dose monitoring of MMF (Jeong and Kaplan 2007). However, subsequent prospective clinical trials showed no significant difference in

biopsy proven acute rejection and graft loss in the fixed dose of MMF compared to concentration controlled arm. Also elevated MPA levels could not be correlated to its toxic effects. With the similar outcomes and extra expenses, routine target dose monitoring has fallen out of favor (Byrne et al. 2011).

6 Side Effects

The relatively rapid cellular turnover in the gastrointestinal tract and bone marrow accounts for their particular susceptibility to the side effects of antiproliferative medications. The most substantial side effect of azathioprine is bone marrow suppression (anemia, thrombocytopenia, and leukopenia), which is why regular monitoring with complete blood counts is essential. Dose reduction may be helpful and improvement in the CBC can be seen as soon as 7–10 days after adjustment (Maltzman and Koretzky 2003). Pancreatitis and hepatotoxicity are less rare but more serious side effects reported with azathioprine (Aarbakke et al. 1997).

As mentioned previously, MPA is more specific for proliferating lymphocytes than azathioprine and as such bone marrow suppression is far less common (Bunnapradist and Ambühl 2008). GI upset, particularly nausea, vomiting, and diarrhea are the most common side effects associated with MPA and may lead to a decreased dosage in many patients. Some studies have shown that utilizing enteric-coated mycophenolate sodium instead of MMF may lead to less GI side effects and higher sustained doses overtime (Ortega et al. 2011).

7 MMF and Azathioprine During Pregnancy

Several prospective and large case series have suggested safety of azathioprine use during pregnancy without increase in malformations (Natekar et al. 2011). This remains one of the indications for use of azathioprine in heart transplantation.

In 2006 the national transplantation pregnancy registry NTPR reported increased risk of both miscarriages and birth defects and female transplant recipients using MPA during pregnancy. This did not appear to affect male recipients. In 2007 the Food and Drug Administration (FDA) issued a black box warning on the use of MPA during pregnancy. Pregnancy testing was also recommended immediately before initiation of MPA and at 8–10 days after use. In order to educate health providers in females receiving the truck with reproductive potentials, the FDA mandated a single shared risk evaluation and mitigation strategies (REMS) system in 2012 (Kim et al. 2013).

8 MMF vs Azathioprine

In the 1990s three pivotal trials compared MMF vs azathioprine based immunosuppression regimens, showing a reduction in incidence of acute rejection post renal transplantation from 40–45% to 20–25% (Placebo-controlled study of mycophenolate mofetil combined with cyclosporin and corticosteroids for prevention of acute rejection. European Mycophenolate Mofetil Cooperative Study Group 1995; A blinded, randomized clinical trial of mycophenolate mofetil for the prevention of acute rejection in cadaveric renal transplantation. The Tricontinental Mycophenolate Mofetil Renal Transplantation Study Group 1996; Sollinger 1995). A few years later the heart transplant community underwent its first large collaboration with a multicenter double-blind, active controlled trial randomizing 650 patients undergoing their first heart transplant to receive azathioprine vs MMF, in combination with cyclosporine and corticosteroids. There were significant reductions in one-year mortality, rejection with hemodynamic compromise and in treatable rejection episodes in the MMF cohort (Kobashigawa et al. 1998).

In an attempt to assess long-term effect of MMF on renal graft survival, the US renal transplant scientific registry was analyzed by Ojo et al. between October 1988 to June 1997 with a total of 66,774 renal transplant recipients, it showed at 4 years MMF reduced the relative risk of graft loss by 27% independent of incidence of acute rejection (Ojo et al. 2000). These trials have led to MMF being the cornerstone as an antiproliferative in solid organ transplant and its replacement of azathioprine.

9 Clinical Trials

Below are some highlighted clinical trials in solid organ transplant with MMF and/or azathioprine (Table 1):

In spite of the numerous clinical trials, there remains no single standardized immunosuppression regimen. Rather, they are individualized based on patient's characteristics, risk profiles, and underlying comorbidities and balancing the risks of over immunosuppression with the risk of rejection (Kobashigawa 2017).

Table 1 Major randomized clinical trials of antiproliferative medications in solid organ transplantation

Study	Comparison	Patients	Follow-up (years)	Survival	Rejection	Notes
Kidney						
European Renal MMF Study group, 1995 (Placebo-controlled study of mycophenolate mofetil combined with cyclosporin and corticosteroids for prevention of acute rejection. European Mycophenolate Mofetil Cooperative Study Group 1995)	MMF 2G vs MMF 3G vs placebo (background of CSA + steroids)	491	0.5	No difference	Less with MMF at both doses (no difference between MMF doses)	
U.S. Renal MMF Study group, 1995 (Sollinger 1995)	MMF 2G vs MMF 3G vs AZA (background of CSA + steroids)	499	0.5	No difference	Less with MMF at both doses (no difference between MMF doses)	Use of antilymphocyte agents for rejection higher in AZA group
Tricontinental Renal MMF Study group, 1995 (A blinded, randomized clinical trial of mycophenolate mofetil for the prevention of acute rejection in cadaveric renal transplantation. The Tricontinental Mycophenolate Mofetil	MMF 2G vs MMF 3G vs AZA (background of CSA + steroids)	503	0.5	No difference	Less with MMF at both doses (no difference between MMF doses)	

Renal Transplantation Study Group 1996)						
Halloran, et al. (meta-analysis), 1997 (Halloran et al. 1997)	MMF 2G vs MMF 3G vs AZA or placebo (background of CSA + steroids)	1,493	1	No difference	Less with MMF at both doses (no difference between MMF doses)	No difference in graft survival between all groups
Shapiro et al. (1999)	TAC vs TAC + MMF (background of steroids)	206	1.5	No difference	Less with TAC + MMF group	
Johnson et al. (2000)	TAC + AZA vs TAC + MMF vs CSA + MMF	223	3	No difference	No difference (though trend towards lower rejection in TAC + MMF group)	CSA + MMF group with significantly worse renal function
Gonwa et al. (Prograf Study Group), 2003 (Gonwa et al. 2003)	TAC + MMF vs TAC + SRL	361	0.5	No difference	No difference	TAC + SRL group with significantly worse renal function
Hall et al. (the ERL B301 Study Group), 2004 (Salvadori et al. 2004)	EC-MPS vs MMF (background of Neoral + steroids)	423	1	No difference	No difference	Both safety profiles and GI adverse events were similar for both groups
MYSS, 2007 (Remuzzi et al. 2007)	MMF + CSA vs AZA + CSA	248	5	No difference	No difference	
Heart						
Kobashigawa et al. (1998)	MMF vs AZA	650	3	Higher with MMF	Less with MMF	Less CAV with MMF at 1 year, but more opportunistic infections
Eisen et al. (2003)	EVR 1.5G vs EVR 3G vs AZA (background of CSA + steroids)	634	1	No difference	No difference	EVR associated with less CAV but worse renal function

(continued)

Table 1 (continued)

Study	Comparison	Patients	Follow-up (years)	Survival	Rejection	Notes
Keogh et al. (2004)	AZA vs SRL	136	2	No difference	Less rejection with SRL at 6 months	SRL associated with less CAV, worse renal function more anemia, AZA with more nausea and arrhythmia
Kobashigawa et al. (2006)	TAC/MMF vs TAC/SRL vs CSA/MMF	343	1	No difference	Less treatable rejection in the TAC groups	TAC/MMF associated with better renal function, TAC/SRL associated with impaired wound healing
TICTAC trial, 2011 (Baran et al. 2011)	TAC vs TAC + MMF (background of early steroids)	150	1	No difference	No difference	No difference in CVA between groups
Eisen et al. (2013)	MMF + standard CSA vs EVR + reduced-dose CSA	721	2	No difference	No difference	EVR with less CAV than MMF, though high-dose EVR (3.0 mg) stopped prematurely due to increased mortality
SCHEDULE trial, 2016 (Andreassen et al. 2016)	MMF + CSA vs EVR + reduced-dose CSA with early CSA withdrawal	115	3	No difference	More mild rejection in EVR-only group, but no difference at 1 year	EVR with less CAV but more opportunistic infections
Liver						
Jain et al. (1998)	TAC vs TAC + MMF (background of steroids)	200	1	No difference	No difference	Trend towards decreased rejection and nephrotoxicity in MMF group

Klupp et al. (1999)	CSA + MMF vs TAC + MMF vs TAC (background of steroids)	120	1.5	TAC + MMF and TAC group with higher survival	TAC + MMF and TAC group with lower rejection	
Fischer et al. (2000)	MMF vs AZA (background of CSA + steroids)	63	1	No difference	Less with MMF	Bone marrow suppression less common with MMF
Wiesner et al. (2001)	MMF vs AZA (background of CSA + steroids)	565	1	No difference	Less with MMF at 6 months, no difference at 1 year	
Lung						
McNeil et al. (2006)	MMF vs AZA (background of CSA + steroids)	315	3	Significant 1 year survival with MMF, not significant at 3 years	No difference	
Strueber et al. (2016)	MMF vs EVR (background of CSA + steroids)	190	2	No difference	Less with EVR	High dropout rate reported in EVR group
Simultaneous pancreas/kidney						
Odorico et al. (1998)	AZA vs MMF (background of CSA + steroids)	109	2	No difference	Less with MMF	Similar reduction in rejection rates for both kidney and pancreas with MMF
Merion et al. (2000)	AZA vs MMF (background of CSA + steroids)	150	0.5	No difference	No difference	

AZA azathioprine, *MMF* mycophenolate mofetil, *EC-MPS* enteric-coated mycophenolate sodium, *CSA* cyclosporine, *EVR* everolimus, *TAC* tacrolimus, *SRL* sirolimus, *CAV* cardiac allograft vasculopathy, *sCr* serum creatinine

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Mechanistic Target of Rapamycin (mTOR) Inhibitors

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Abstract

Mechanistic target of rapamycin (mTOR) inhibitors are macrocyclic lactone antibiotics derived from *Streptomyces hygroscopicus* that prevent T lymphocyte activation and B cell differentiation. Unlike calcineurin inhibitors (CNIs) that inhibit cytokine production, mTOR inhibitors block the cytokine signal transduction to arrest cells in the G1 to S phase. This class of drugs is commonly used for post-transplantation and cancer management because of its immunosuppressive and antiproliferative properties, respectively. The potential uses of mTOR inhibitors are heavily explored because of their impact on cell growth and proliferation. However, mTOR inhibitors have a broad range of effects that can result in adverse reactions, but side effects can occur with other immunosuppressive agents as well. Thus, the performance of mTOR inhibitors is compared to the outcomes and adverse effects of other immunosuppressive drugs or the combination of other immunosuppressants and mTOR inhibitors. Because mTOR regulates many downstream pathways, mTOR inhibitors can affect these pathways to manage various diseases. Sirolimus (rapamycin) is approved by the Food and Drug Administration (FDA) to treat post-renal transplantation and lymphangioleiomyomatosis (LAM). Everolimus is approved by the FDA to treat postmenopausal advanced hormone receptor-positive, HER2-negative breast cancer in women, progressive neuroendocrine tumors of pancreatic origin (PNET), advanced renal cell carcinoma (RCC), renal angiomyolipoma (AML) and tuberous sclerosis complex (TSC), and subependymal giant cell astrocytoma (SEGA) associated with TSC as well as renal and liver transplantation. Temsirolimus is approved by the FDA to treat advanced RCC. Opportunities to use mTOR inhibitors as therapy for other transplantation, metabolic disease, and cancer management are being researched. mTOR inhibitors are often called proliferation signal inhibitors (PSIs) because of their effects on proliferation pathways.

Keywords

Cancer immunosuppression · Graft rejection treatment · Proliferation signal inhibitors · Transplantation immunosuppression

1 Introduction

Induction therapy for kidney transplantation historically used high dose corticosteroids in combination with T cell-directed therapy, which included antibodies targeting thymocyte globulin, IL-2 receptor, CD3, and CD52. Such immunosuppressants are used to prevent acute rejection. Later, other agents were discovered for maintenance immunosuppression to extend the life of the graft. CNIs (tacrolimus, cyclosporine), antiproliferative agents (mycophenolate mofetil, azathioprine), and mTOR inhibitors (sirolimus and everolimus) are common classes of maintenance drugs. These immunosuppressants are considered more potent than

those that were previously used. Sirolimus (SRL) specifically is an advantageous immunosuppressant for post-kidney transplantation maintenance therapy because of its low nephrotoxicity (Sabbatini et al. 2000). It is metabolized by the liver and has a low impact on renal function. It is mainly excreted in feces (91%) and a minor amount excreted in urine (2.2%) (Product Information 2012, 2018). With such an advantage, therapies with mTOR inhibitors are compared to those without mTOR inhibitors in search of better morbidity and mortality outcomes for patients.

The success of mTOR inhibitors as immunosuppressants in kidney transplantation therapy led to the investigation of their use in other immunosuppressive therapies. They are used in other transplant therapies, like heart transplantation, and related comorbidities are evaluated. Studies focus on optimal outcomes with minimal immunosuppressants to limit the burden of drug interactions and adverse reactions. Research analyzed the results of the conversion from a CNI to SRL, combination of other immunosuppressants with mTOR inhibitors, mTOR inhibitors used in maintenance therapy, and comparison of mTOR inhibitors to other immunosuppressants on severity of side effects due to duration and dosage. These studies further the understanding of mTOR-dependent pathways, the mechanism of action of mTOR inhibitors, and other uses of mTOR inhibitors. Exploration of additional targets that complement the effects of mTOR inhibitors can synergistically improve outcomes.

The mTOR inhibitors branched out from transplantation therapies into other disciplines. They are used to prevent restenosis after coronary artery stent placements and used in tumor treatments of TSC, RCC, PNET, LAM, and breast cancer (Sehgal 2003; Product Information 2012, 2018). Most of these indications leverage the antiproliferative properties of mTOR inhibitors to regress tumors (Sehgal 2003). However, many of the tumors regrow after withdrawal of mTOR inhibitors, uncovering their cytostatic traits and leading to studies centered around the long-term effects of mTOR inhibitors (Bissler et al. 2013).

2 Mechanism of Action

2.1 mTOR

Mechanistic target of rapamycin is a threonine/serine protein kinase within the PIKK family. It has two major complexes, mTOR complex 1 (mTORC1) and mTOR complex 2 (mTORC2), with distinct functions to regulate cellular processes (Hara et al. 2002; Kim et al. 2002; Sarbassov et al. 2004). The mTORC1 receives signals from growth factors, energy, nutrients, and oxygen to modulate cell growth and proliferation (Kim et al. 2002). The mTORC2 receives signals to regulate cell survival and actin organization in the cytoskeleton (Laplante and Sabatini 2012). The system has multiple feedback loops for cell survival.

The mTORC1 is comprised of mammalian lethal with sec-13 protein 8 (mLST8), regulatory associated protein of TOR (raptor), DEP-domain containing mTOR interacting protein (DEPTOR), and proline-rick Akt substrate 40 kDa (PRAS40)

(Hara et al. 2002; Sarbassov et al. 2004). DEPTOR and raptor are regulatory components of mTORC1. When substrates eukaryotic elongation factor 4E-binding protein (4E-BP1) and S6 ribosomal protein kinase (S6K) are phosphorylated, translation is initiated to regulate important cellular functions, such as protein synthesis, lipid synthesis, nucleotide synthesis, glycolysis, and autophagy (Ma and Blenis 2009; Jewell and Guan 2013). For instance, activation of mTORC1 stimulates glycolysis, lipid synthesis, and glutamine metabolism through hypoxia-inducible factor alpha (HIF1α) and c-Myc, sterol regulatory element binding protein (SREBP-1), and SIRT4 repression, respectively (Yecies and Manning 2011). Since mTORC1 is downstream in the PI3K/Akt pathway for tumor suppressors (PTEN, LKB1, TSC1/2, PI3K, Akt), its signaling changes can alter metabolic synthesis and turnover and autophagy repression (Laplante and Sabatini 2012; Jewell and Guan 2013). Disturbances to the regulation of the mTORC1 pathway have been associated with genetic disorders, uncontrolled cell growth, and aging and aging-related diseases (Johnson et al. 2013a).

The mTORC2 has mTOR rapamycin insensitive companion of mTOR (rictor), stress-activated protein kinase-interacting protein 1 (MSIN1), and mLST8. By activating Akt and SGK1, mTORC2 drives cell survival (Huang and Manning 2009). With the activation of PKCα, paxillin, and small GTPases (Rho and Rac), mTORC2 regulates actin organization of the cytoskeleton (Laplante and Sabatini 2012).

2.2 Rapamycin

Rapamycin binds FKBP-12, a cytosolic protein, to form a complex that interacts with mTOR to prevent cytokine (IL-2, IL-4, IL-15) signaling (Benjamin et al. 2011; Hardinger et al. 2004). Rapamycin preferentially inhibits mTORC1 initially, but it inhibits mTORC2 after long incubation in some cells (Huang and Manning 2009). Thus, its use in continuous immunosuppressive therapies usually results in inhibition of both mTORC1 and mTORC2. The treatment duration does not only affect rapamycin's differential inhibition between mTORC1 and mTORC2. Short-term treatment of rapamycin dephosphorylates 4E-BP1, but it phosphorylates 4E-BP1 if treatment lasts longer than 12 h (Choo et al. 2008). Rapamycin inhibits S6K of mTORC1, which removes the repression on autophagy (Jewell and Guan 2013). Yet, this block triggers a feedback loop. Decreased PI3K/Akt upstream signaling increases receptor tyrosine kinases (RTK), PI3K/Akt, and Ras-ERK activity that will increase PI3K/Akt pathway activity for cell survival (Benjamin et al. 2011). The combined effects of rapamycin on 4E-BP1 and S6K produce cap-dependent translation (Choo et al. 2008). The decreasing downstream signaling induces stress, reduces protein synthesis, and induces autophagy, while the increasing upstream signaling for cell survival results in the cytostatic, not cytotoxic, effects of rapamycin (Bissler et al. 2013). The poor efficacy of rapamycin comes from the phosphorylation of T37 and T47 of 4E-BP1 that are rapamycin resistant, the weak phosphorylation of T389 of S6K1 that is rapamycin sensitive, and the alteration of the substrate

phosphorylation site that is sensitive to rapamycin and other mTORC1 signals (growth factors, nutrients) (Thoreen and Sabatini 2009). Such findings have led to closer examination of ATP-competitive mTOR inhibitors to block mTORC1 and mTORC2 to prevent PI3K/Akt activation in hopes of more potent and sustained effects of mTOR inhibitors (Benjamin et al. 2011).

2.3 Rapamycin Analogs

Temsirolimus and everolimus are rapamycin analogs (rapalogs) approved by the FDA. These sirolimus derivatives have similar mechanisms of action to rapamycin. However, everolimus preferentially inhibits mTORC1 without mTORC2 inhibition, while temsirolimus is a prodrug of sirolimus (Product Information 2012). Everolimus inhibits HIF-1 and reduces vascular endothelial growth factor (VEGF) expressions that lead to reduced cell proliferation, angiogenesis, and glucose uptake (Product Information 2012). It prevents S6K1 from phosphorylating the activation domain 1 of the estrogen receptor, which has gotten everolimus FDA approval for treatment of postmenopausal advanced hormone receptor-positive, HER2-negative breast cancer in women (Product Information 2012). Similar to everolimus, temsirolimus reduces HIF-1, HIF-2α, and VEGF (Product Information 2011).

2.4 Pharmacokinetics

Sirolimus reaches peak concentration (T_{max}) in 1 h in healthy subjects and 2 h in renal transplant patients (Product Information 2018). It has poor solubility and a low bioavailability of 14% with the oral solution (Product Information 2018). Sirolimus concentration is not immensely affected by food, except for grapefruit. Therefore, it is recommended to take sirolimus consistently at the same time daily. Since it is a substrate of CYP3A4 and P-gp, inhibitors and inducers of CYP3A4 and P-gp need to be taken with caution in combination with sirolimus. Co-administration of inhibitors or inducers of CYP3A4 and P-gp can change the concentration of sirolimus.

3 Clinical Trials

The mTOR inhibitors have clinical trials that established their appropriate indications. Some of the trials focus on expansion of their uses, while others compare their outcomes to those of other immunosuppressants. Specifically, several measure CNIs against mTOR inhibitors because they influence similar pathways.

3.1 SMART Trial

One of the more well-known trials is the SMART trial, a randomized trial that summarized the outcomes of early conversion from cyclosporin A (CsA) to SRL post-renal transplantation (Guba et al. 2010). The study identified 198 potential participants, but only 141 patients met the criteria. Most were excluded due to surgical/wound complications ($n = 22$) or unresolved rejection ($n = 16$). All 141 subjects received antithymocyte globulin-F (ATG) single-bolus induction, mycophenolate mofetil (MMF), and steroids. Participants either stayed on CsA or converted to SRL within 10–24 days post-renal transplantation and were followed for a year. Of the 70 allocated to the treatment group, 69 were treated with SRL, MMF, and steroids. The SRL group had 44 complete the treatment, 25 discontinued the treatment, and 3 lost to follow-up. The control group had 71 allocated and received treatment of CsA, MMF, and steroids. The CsA group had 57 complete the treatment, 14 discontinued the treatment, and 2 lost to follow-up. The study found that S-creatinine and eGFR were better in the SRL group than the CsA group (1.51 ± 0.59 vs. 1.87 ± 0.98 mg/dL and 64.5 ± 25.2 vs. 53.4 ± 18.0 mL/min/1.73 m^2). No statistical significance was found for patient survival, graft survival, and incidence of biopsy-proven acute rejection after conversion. Treatment discontinuation was significantly higher in SRL group and mainly related to adverse events (36.2% vs. 19.7%). The SRL group also had significantly more side effects of acne, aphthous, and temporary hyperlipidemia, but decreased cytomegalovirus viremia (7.3% vs. 28.2%). Thus, the SMART trial suggests that early conversion from CsA to SRL may improve renal function for patients at low-to-moderate immunological risk and acceptable adverse event rates. Further trials have been conducted to examine starting immunosuppression with mTOR inhibitors and without a CNI, or using mTOR inhibitors with or to replace other immunosuppressants of different targets.

3.2 EXIST Trials

The EXIST trials examine the outcomes of everolimus used for the treatment of TSC or TSC-associated effects. The EXIST-1 trial is a double-blind, randomized, controlled study analyzing the efficacy and safety of everolimus for the use of subependymal giant cell astrocytomas (SEGA) associated with TSC (Franz et al. 2013). It measured everolimus impact on SEGA-associated TSC, adverse events, and other associated findings. The 117 participants continued their TSC treatments and were randomly allocated to the treatment group that received everolimus ($n = 78$) or placebo group that received no additional treatment ($n = 39$) for SEGA-associated TSC. The everolimus group lost 1 to follow-up, 1 to discontinued treatment, and 1 to withdrawal of consent, while the placebo group lost 6 to disease progression, 8 to discontinued treatment, 1 to withdrawal of consent, and 1 to administrative problems. Of the 78 on everolimus, 27 (35%) had at least 50% reduction in SEGA volume versus no reduction in volume in the placebo group

(difference 35%, 95% CI 15–52; one-sided exact Cochran–Mantel–Haenszel test, $p < 0{\cdot}0001$). MRI detected the reduction by 12 weeks, and such responses were seen for 63–255 days. Reductions in comorbid skin lesions and kidney tumors were observed in those on everolimus as well. Though some of those on everolimus experienced adverse events, such as mouth ulceration and stomatitis, none required discontinuation of treatment. EXIST-1 trial established the efficacy and safety of everolimus for treatment of SEGA associated with TSC, which prompted additional trials to examine using everolimus for managing other TSC-associated effects.

EXIST-2 trial analyzes the efficacy and safety of using everolimus to treat AML associated with TSC or sporadic lymphangioleiomyomatosis (sLAM) (Bissler et al. 2015). Of the 118 patients, 79 received everolimus and 39 received a placebo. Two patients on everolimus had to discontinue treatment due to adverse events, while 13 patients on placebo had to discontinue treatment due to disease progression ($n = 9$) or adverse events ($n = 4$). The most common adverse events for both groups were stomatitis, nasopharyngitis, and acne-like skin lesions. The AML response rate (reduction in total volume) was 42% (33 of 79 [95% CI 31–53%]) for everolimus and 0% (0 of 39 [0–9%]) for placebo (response rate difference 42% [24–58%]; one-sided Cochran-Mantel-Haenszel test $p < 0{\cdot}0001$). Those on everolimus not only experienced AML shrinkage, but they did not experience hemorrhage that is common within such a patient population.

EXIST-3 trial studies the effects of everolimus of treatment-resistant focal-onset seizures associated with TSC (French et al. 2016). Patients were on 1–3 antiepileptic drugs and were stratified by age and randomized into three groups: low exposure of everolimus (3–7 ng/mL; $n = 117$), high exposure of everolimus (9–15 ng/mL; $n = 130$), and placebo ($n = 119$). The trial defined response rate as proportion of patients with $\geq$50% reduced seizure frequency. The placebo group had a response rate of 15.1% (95% CI 9.2–22.8; 18 patients); low exposure group had a response rate of 28.2% (95% CI 20.3–37.3; 33 patients; $p = 0.0077$); and high exposure group had a response rate of 40.0% (95% CI 31.5–49.0; 52 patients; $p < 0001$). The median percentage reduction in seizure frequency was 14.9% for placebo, 29.3% for low exposure ($p = 00.28$), and 39.6% for high exposure ($p < 0.0001$). There were more adverse events with higher doses of everolimus. Some were serious enough that patients had to discontinue treatment: 2% ($n = 2$) in the placebo group, 5% ($n = 6$) in the low exposure group, and 3% ($n = 4$) in the high exposure group. Additional reasons for discontinuing treatment included consent withdrawal ($n = 1$ for placebo; $n = 2$ for low exposure; $n = 1$ for high exposure), lack of efficacy ($n = 2$ for placebo; $n = 2$ for high exposure), and protocol deviation ($n = 1$ for high exposure).

The EXIST trials established efficacy and safety for everolimus for TSC-associated effects and examined the impact of varying dosages. They lead to the potential exploration of using mTOR inhibitors to manage symptoms or effects of other grievous illnesses.

3.3 BOLERO Trials

The BOLERO trials test the efficacy and safety of everolimus for the use of treating advanced breast cancer. The series of trials analyzes the effects of everolimus when combined with current treatment agents of breast cancer.

BOLERO-1 trial specifically examines the effects of adding everolimus to the treatment of postmenopausal women with HER2+ advanced breast cancer (Toi et al. 2017). It demonstrated the benefits of everolimus for this indication and further explored them for the Asian subpopulation. All patients received trastuzumab and paclitaxel. They were stratified by Asian and non-Asian and randomized 2:1 to have the addition of everolimus or placebo to this regimen. BOLERO-1 trial measured the progression-free survival (PFS), response rate, clinical benefit rate, and safety. Within the Asian, the everolimus ($n = 198$) and placebo ($n = 105$) arms were found to have similar median PFS (hazard ratio = 0.82; 95% CI 0.61–1.11), but everolimus (median PFS 25.46 months) prolonged the median PFS by 10.97 months when compared to placebo (median PFS 14.49 months) for patients with hormone receptor-negative (HR-) breast cancer (hazard ratio = 0.48; 95% CI 0.29–0.79). Thus, the PFS benefit for the Asian subset was seen for the HR-subgroup but not seen for the HER2+ subgroup. Everolimus had adverse events with the most serious ones being pneumonia, pneumonitis, and interstitial lung disease. Decreased neutrophil count and leukopenia had higher incidences in the Asian subgroup regardless of the arm they were randomized into when compared to the non-Asian subgroup. During the duration of the study, three deaths were within the Asian everolimus arm due to disease progression, pneumonia, or sepsis, while the non-Asian everolimus arm had 19 deaths. Compared to the placebo arm, no deaths were within the Asian subpopulation and two deaths were within the non-Asian population. The study found some differences between the Asian and non-Asian subsets, such as greater PFS benefit in the Asian subset using everolimus for those who were previously treated with taxane, had HR-disease, had disease relapse <24 months after diagnosis, or had no bone involvement. However, the Asian subset had a longer duration of all agents (trastuzumab, paclitaxel, and everolimus) and had everolimus at a lower dose than the non-Asian subset. Such outcomes resulted in further investigation of pairing everolimus to other breast cancer treatments.

BOLERO-2 trial examines the addition of everolimus to exemestane for the treatment of postmenopausal HR+ advanced breast cancer (Beaver and Park 2012). Similar to BOLERO-1 trial, BOLERO-2 trial is measuring the PFS and interaction to a current treatment of breast cancer. All patients received exemestane and were randomized 2:1 into the everolimus ($n = 485$) or placebo ($n = 239$) group. The median PFS was 6.9 months for the everolimus group versus 2.8 months for the placebo group (hazard ratio: 0.43; $p < 0.001$). The response rate for the everolimus and exemestane treatment was approximately 10%, which was similar to the results of single-agent temsirolimus and some single-agent multitargeted tyrosine kinase inhibitors. Though everolimus arm had more grade 3 and 4 adverse events than the placebo arm, the quality of life and Eastern Cooperative Oncology Group (ECOG) status did not have a significant difference between the two groups. Common

adverse events in the everolimus group included stomatitis, anemia, fatigue, and pneumonitis.

BOLERO-3 trial investigates the results of everolimus when used with trastuzumab and vinorelbine for HER2+, trastuzumab-resistant advanced breast cancer patients who previously received taxane treatment (André et al. 2014). The trial studied similar parameters to prior BOLERO trials with the additional assessment of whether everolimus can restore sensitivity to trastuzumab. All patients ($n = 569$) received trastuzumab and vinorelbine. They were randomized to add everolimus ($n = 284$) or a placebo ($n = 285$) and stratified for prior lapatinib therapy. The median PFS was 7.00 months and 5.78 months for everolimus (95% CI 6.74–8.18) and placebo (hazard ratio 0.78; 95% CI 0.65–0.95; $p = 0.0067$), respectively. Though the findings suggest that everolimus addition significantly prolongs PFS in HER2+, trastuzumab-resistant, taxane-pretreated advanced breast cancer patients, 42% ($n = 117$) had serious adverse events in the everolimus group compared to 20% ($n = 55$) in the placebo group. The most common adverse events were neutropenia, leukopenia, anemia, febrile neutropenia, stomatitis, and fatigue. Therefore, the benefits of using everolimus should be weighed against the potential risks.

3.4 RECORD Trials

An indication for mTOR inhibitors, such as temsirolimus and everolimus, is metastatic or advanced RCC. The RECORD trials focus on the use of everolimus for this indication and analyze its efficacy and safety.

The RECORD-1 trial tests the effects of everolimus in patients with metastatic RCC who still progressed with vascular endothelial growth factor (VEGF) therapy (Motzer et al. 2008). All the patients had metastatic RCC that progressed on sunitinib, sorafenib, or both and were randomized 2:1 to add everolimus ($n = 272$) or placebo ($n = 138$). The study measured PFS and planned to terminate after 290 progression events. Because of the significant difference in efficacy, the trial ended after 191 progression events; the 37% ($n = 101$) and 65% ($n = 90$) progressive events occurred in the everolimus and placebo groups (hazard ratio 0.30; 95% CI 0.22–0.40; $p < 0{\cdot}0001$), respectively. The median PFS was 4.0 months (95% CI 3.7–5.5) for the everolimus arm and 1.9 months (95% CI 1.8–1.9) for the placebo arm. Adverse events were observed more frequently in the everolimus group, with the most common ones being stomatitis, rash, and fatigue. RECORD-1 trial demonstrated that everolimus can prolong PFS when compared to placebo and led to further studies on outcomes of everolimus in addition to other RCC treatment methods.

RECORD-2 trial compared everolimus and bevacizumab treatment against interferon α-2a and bevacizumab treatment for first-line therapy of metastatic RCC (Ravaud et al. 2015). Untreated patients were randomized 1:1 to receive everolimus and bevacizumab ($n = 182$) or interferon and bevacizumab ($n = 183$). The study found that the treatment results were comparable. The median PFS was 9.3 months

for everolimus group versus 10 months for interferon group ($p = 0.485$). The median duration of exposures was also similar with 8.5 months for everolimus arm and 8.3 months for interferon arm. RECORD-2 trial showed similar adverse events, but with the addition of proteinuria (33% in everolimus and bevacizumab, 18.8% in interferon and bevacizumab). Such outcomes suggest that everolimus can potentially be an alternative to interferon in first-line therapy of metastatic RCC.

Everolimus was further tested against other first-line therapies in RECORD-3 trial, which compared using everolimus followed by sunitinib ($n = 238$) to using sunitinib followed by everolimus ($n = 233$) for metastatic RCC (Knox et al. 2017). The overall survival analysis showed that sunitinib followed by everolimus had better outcomes. The median duration of exposure was 5.6 months for everolimus and 8.3 months for sunitinib. The median overall survival was 22.4 and 29.5 months for everolimus followed by sunitinib and sunitinib followed by everolimus (hazard ratio: 1.1; 95% CI 0.9–1.4), respectively. For both arms, the most common reasons for inability to start second-line treatment were ineligibility associated with poor performance status or condition decline, death, and consent withdrawal. The study also found that elevated neutrophil lymphocyte rate and 12 soluble biomarkers were associated with poor overall survival regardless of the treatment sequence. Thus, future research may include stratifying these factors.

A RECORD-4 trial was done to test the efficacy and safety of second-line everolimus in Asian and non-Asian populations for metastatic RCC (Yang et al. 2018). Patients were previously on anti-VEGF or cytokine therapies. The PFS was similar between the Asian (74.5%; 95% CI 61.0–85.3) and non-Asian (74.7%; 95% CI 63.6–83.8) groups.

4 Clinical Uses

Mechanistic target of rapamycin inhibitors have a variety of uses given the pathways that they impact. Not only are they commonly used in transplantation and cancer regimens, but current research is also analyzing their potential for metabolic and neurological uses.

4.1 Kidney Transplantation

Kidney transplantation was the first indication for mTOR inhibitors, mainly for maintenance therapy to avoid rejection. They are generally combined with or replacing other classes of immunosuppressants, such as steroids, CsA, azathioprine (AZA), tacrolimus, and MMF. For instance, mTOR inhibitors are an alternative option for patients who have CNI toxicity or are noncompliant with therapy. The benefits of using mTOR inhibitors for kidney transplantation therapy include lower rates of cytomegalovirus (CMV) infection than other immunosuppressive agents and lower rates of non-melanoma skin cancer than CsA (Ghassemieh et al. 2013; Andrassy et al. 2012; Nashan et al. 2012; Euvrard et al. 2012). They can treat post-renal transplantation Kaposi sarcoma, refractory kidney transplant rejection,

and chronic renal allograft nephropathy (Stallone et al. 2005; Hong and Kahan 2001; Flechner et al. 2004). They help ease patients through steroid withdrawal to decrease glucocorticoid-induced morbidity (Matas et al. 2005). However, there are potential risks that accompany the use of mTOR inhibitors. A systematic review and meta-analysis study found that SRL was associated with greater mortality than non-SRL immunosuppressive regimens for kidney and pancreas-kidney transplantations (Knoll et al. 2014). Though they are associated with lower rates of non-melanoma skin cancer, they have not yet demonstrated the same results for melanoma (Knoll et al. 2014; Yanik et al. 2015; Lim et al. 2014). Sirolimus is associated with delayed allograft function, poor wound healing, adverse short-term effects, and increased lymphoceles (Product Information 2018). Thus, transplantation therapy needs to be tailored to each patient to balance the advantages and risks of the different immunosuppressants.

4.2 Heart Transplantation

Similar to kidney transplantation, heart transplantation uses mTOR inhibitors for maintenance immunosuppression. They are not generally used during the early post-transplantation period since using mTOR inhibitors within the first week post-transplantation has been associated with significant renal dysfunction (Kobashigawa et al. 2013; Eisen et al. 2003, 2013; Keogh et al. 2004). However, heart transplantation patients who convert early from a CNI to SRL seem to have increase survival, attenuated CAV progression, lower all-cause mortality, and fewer CAV-related events (Asleh et al. 2017). Furthermore, patients who develop renal dysfunction with CNIs improve after switching to an mTOR inhibitor (Gustafsson et al. 2007; Raichlin et al. 2007; Bestetti et al. 2006; Fernandez-Valls et al. 2005; Kushwaha et al. 2005; Hunt et al. 2005; Groetzner et al. 2004). Specifically, SRL had a lower proportion of biopsy-proven moderate to severe acute cellular rejection at 6 months when compared to AZA at both 3 mg/day ($p = 0.027$) and 5 mg/day ($p = 0.013$) (Keogh et al. 2004). Everolimus had similar performance to MMF for biopsy-proven acute cellular rejection, acute rejection with hemodynamic compromise, and graft loss (Eisen et al. 2013). Everolimus had superior results for reduced progression of intimal wall thickening of the coronary artery (Eisen et al. 2013). However, everolimus had more adverse events, such as pericardial effusions, than MMF (Eisen et al. 2013). Therefore, assessment of the relative risks of the patient is required to determine if everolimus is preferable.

An alternative approach to using everolimus is the early replacement CNI with everolimus as was accomplished in the SCHEDULE clinical trial (Arora et al. 2018). The authors in this Scandinavian randomized study showed that CNI could be replaced with everolimus from 7 to 11 weeks post-transplant with attenuation of cardiac allograft vasculopathy assessed by IVUS and with preservation and improvement in renal function in the everolimus group. The only down side appeared to be several patients in the everolimus group had several ISHLT Grade 2R rejections requiring addition of CNIs. However, this was a small minority of the everolimus patients.

A more recent clinical trial, the MANDELA trial, was a randomized, open label, parallel-group study in which renal function, efficacy defined as prevention of biopsy-proven rejection (BPAR), and safety were determined in 200 heart transplant patients (Barten et al. 2019). In the first 3–6 months post-transplant, patients received calcineurin inhibitors (CNI, either tacrolimus or cyclosporine), corticosteroids and either the mTOR inhibitor everolimus or mycophenolate mofetil (MMF). At 6 months, the patients were randomized to either a CNI-free regimen with everolimus and MMF or a low-dose CNI regimen with everolimus. Patients were then followed for an additional 18 months. At 18 months, renal function defined by GFR was improved in the CNI-free group vs. the reduced dose CNI/everolimus group. However, BPAR was significantly less frequent in the reduced dose CNI/everolimus group than in the CNI-free group. Renal function was acceptable and improved as the CNI dose was reduced in the reduced dose/CNI group.

The Mayo Clinic has developed a novel approach to the use of mTOR inhibitors by switching patients from CNI-based immunosuppression to CNI-free, mTOR inhibitor (sirolimus) at one 1–2 years post-transplant. They demonstrated that those switched at 1 year had a reduction in Cardiac Allograft Vasculopathy (CAV) progression defined by intravascular ultrasound and also demonstrated improved survival and reduced incidence of CAV-related events. In that pivotal study, 268 of 402 patients were converted from CNI to sirolimus and 235 had sequential IVUS to assess CAV progression. They were compared to 134 patients treated with CNI of whom 99 had sequential IVUS studies (Asleh et al. 2018; Eisen et al. 2018). One concerning signal from this group's research was that heart transplant patients who developed significant proteinuria after initiation of mTOR inhibitors had increased all-cause mortality and those with proteinuria had a higher incidence of hypertension, and had increased serum creatinines and decreased renal function as defined by eGFRs (Asleh et al. 2020).

4.3 Tuberous Sclerosis Complex

Since mTOR inhibitors have antiproliferative properties, they inhibit cell proliferation. Such a feature is helpful in regulating growth in TSC and is explored in the EXIST trials. TSC results from a loss of function mutation in the TSC genes, which results in release of mTORC1 inhibition (Menon and Manning 2008). Epilepsy, seizures, neurocognitive dysfunction, and autism associated with TSC improved with the administration of mTOR inhibitors (French et al. 2016; Bové et al. 2011). Through mTORC1 inhibition, they seem to have some neuroprotective properties, possibly from blocking the accumulation of misfolded and aggregated proteins driven by protein synthesis and defective autophagic degradation of mTORC1 activity (Bové et al. 2011). Neuroprotection was seen in mice in vivo models for neurodegenerative diseases, such as Alzheimer disease, Huntington disease, Parkinson disease, and spinocerebellar ataxia type 3 (Bové et al. 2011). Rapamycin improves spatial learning and memory impairments, but it worsens exploratory activity in mice (Neff et al. 2013). Rapamycin has also been shown to improve

survival and attenuate Leigh syndrome in mice (Johnson et al. 2013b). Both SRL and everolimus have been shown to decrease AML volume associated with TSC, but the effects were temporary (Bissler et al. 2008, 2013). When SRL was discontinued, the tumors grew back to their original size, highlighting the cytostatic effects of mTOR inhibitors (Bissler et al. 2008). Research on long-term use of mTOR inhibitors for TSC-associated diseases can help evaluate if constant suppression of growth outweighs the risks associated with mTOR inhibitor therapy.

4.4 Lymphangioleiomyomatosis

LAM is a rare lung disease that arises from abnormal proliferation of smooth muscle-like cells. Though LAM is considered a benign metastasis, it can cause cysts, an anti-inflammatory response, and respiratory failure (Yu and Henske 2010). LAM tends to occur in women of reproductive age sporadically or associated with TSC. A randomized controlled trial placed patients on SRL ($n = 46$) or placebo ($n = 43$) and found forced expiratory volume in 1 s (FEV_1) improved with SRL (1 ± 2 mL/month; $p < 0.001$) versus placebo (-12 ± 2 mL/month) (McCormack 2011). The SRL group also had improved forced vital capacity, functional residual capacity, serum vascular endothelial growth factor D, and quality of life and functional performance when compared to the placebo group. However, lung function started to deteriorate again after SRL was discontinued. Not only are long-term consequences of mTOR inhibitors use for the treatment of LAM is being examined, but therapies of ATP-competitive mTOR inhibitors or in combination with estrogen antagonists are also being pursued.

4.5 Cancer

Since mTOR inhibitors target pathways involved in cell growth and proliferation, the class is a potential option for cancer regimens. Even though they are used for some cancer indications, most studies find that they are not as effective as anticipated and are generally used in combination with other therapies. One theory on the limitation of mTOR inhibitors in cancer treatments hypothesizes that genetic variation can cause de novo resistance (Carew et al. 2011). Additionally, mTOR inhibitors display cytostatic, not cytotoxic, properties since other regulators can drive cell survival even when mTOR inhibitors are used (Bissler et al. 2013). Their modest efficacy stems from their inability to completely block mTORC1-mediated signaling, which has multiple feedback loops and compensatory pathways that promote survival and growth (Thoreen and Sabatini 2009). They halt the growth of tumors, but do not permanently stop tumor growth. Thus, they specifically have been found to not be effective alone in major solid tumors. Research is currently being conducted on the effects of using mTOR inhibitors long term to prolong the cytostatic state. An alternative approach to overcome their lack of cytotoxic nature is to pair them with cytotoxic agents, such as various chemotherapies or hormonal

therapies. The addition of mTOR inhibitors can lower the dosage of cytotoxic agents needed, which may reduce side effects. The BOLERO and RECORD trials are studies that have shown that this method is effective. Thus, mTOR inhibitors have been used with aromatase inhibitors, taxanes, and growth factor inhibitors. Studies are investigating the efficacy of combining mTOR inhibitors with other potential therapies as well. Dual inhibitors, those that inhibit mTORC1 and mTORC2, have demonstrated better results than selective inhibitors for cancer (Pallet and Legendre 2012). Though, dual inhibitors can dampen AKT's pro-survival function, they are also more toxic (Shor et al. 2009). The advantages of dual inhibitors may not outweigh the risks. Beyond the indications that the FDA has approved, EU has approved temsirolimus for neuroendocrine tumors, advanced and recurring endometrial cancer, and refractory mantle cell lymphoma. Trials for everolimus to be used for advanced gastric cancer, advanced hepatocellular carcinoma, and non-small cell lung cancer are underway. Ridaforolimus is a rapamycin analog that has shown promising results for advanced bone and soft-tissue sarcomas (Wander et al. 2011).

4.6 Metabolic Diseases

Though mTOR inhibitors are not used for metabolic diseases, there are studies that analyze their outcomes. Dysregulation of mTOR signaling has been found in metabolic diseases, such as diabetes and obesity (Laplante and Sabatini 2012). The findings for the use of mTOR inhibitors on metabolic syndromes have been contradictory. Acute administration of rapamycin improves insulin sensitivity by disrupting the S6K-mediated feedback loop (Krebs et al. 2007; Tremblay and Marette 2001). Mechanistic target of rapamycin inhibitors inhibit human adipocyte differentiation that protects against high fat diet-induced obesity in specific strains (Bell et al. 2000). Yet, patients on mTOR inhibitors experience worsened diabetes mellitus type 2, hyperglycemia, glucose intolerance in diet-induced obesity, hyperlipidemia, and insulin resistance (Product Information 2011, 2012, 2018). Duration of exposure and transient versus permanent effects may contribute to the different results.

4.7 Adverse Reactions

There are many side effects with using mTOR inhibitors since mTOR regulates many pathways. Some of the effects are temporary, permanent, or dose-related. Patients may develop thrombocytopenia, but studies have shown that it normalizes in approximately 2 weeks (Murgia et al. 1996). Leukopenia can develop and is not dose-dependent, but it is reversible with the discontinuation of mTOR inhibitors (Murgia et al. 1996). Hemolytic uremic syndrome and thrombotic thrombocytopenic purpura are associated with the use of SRL with CsA more than other immunosuppressive regimens (Kaplan et al. 1998). Proteinuria and focal segmental glomerulosclerosis have been observed in patients on SRL with CsA (Letavernier

et al. 2005, 2007; Dittrich et al. 2004; Sennesael et al. 2005). Proteinuria is not detected after the SRL withdrawal (Letavernier et al. 2005). Because of its immunosuppressive qualities, patients should be monitored for fatigue, pallor, fever, infection, bruising, and bleeding (Product Information 2011, 2012, 2018). Hyperlipidemia and hypercholesterolemia are dose-dependent. Sirolimus and everolimus have been found to inhibit lipoprotein lipase (Kasiske et al. 2008; Kraemer et al. 1998). Long-term use of SRL is associated with new onset of diabetes (Sulanc et al. 2005; Johnston et al. 2008). Other gastrointestinal issues include nausea, vomiting, diarrhea, constipation, dyspepsia, non-herpes simplex virus mouth sores, and oral ulcers (Product Information 2011, 2012, 2018). These GI symptoms are alleviated with the discontinuation of mTOR inhibitors (Product Information 2011, 2012, 2018). Patients with hepatic impairment should use 1/3 of the recommended dosage of SRL and ½ of the recommended dosage of everolimus (Product Information 2012, 2018). Peripheral edema does not always resolve with discontinuation of mTOR inhibitors (Product Information 2011, 2012, 2018). Other cutaneous adverse reactions include acne, scalp folliculitis, angioedema, impaired wound healing, and epistaxis (Product Information 2011, 2012, 2018). Patients recover from certain pulmonary complications, such as lymphocytic alveolitis and bronchiolitis obliterans organizing pneumonia, within 6 months of SRL discontinuation (Product Information 2018; Champion et al. 2006; Weiner et al. 2007). Liver transplantation patients are not recommended to use mTOR inhibitors because of increased risk of hepatic artery thrombosis and graft loss (Product Information 2018). When combined with a CNI, there is an increased risk of death with liver transplantation patients (Product Information 2018). Pericardial effusion is associated with sirolimus in cardiac and kidney transplantation (Product Information 2011, 2018; Steele et al. 2008).

5 Drug Interactions

Since mTOR inhibitors are metabolized by cytochrome P450, dosage adjustments are necessary for cytochrome P450 inducers and inhibitors (Product Information 2011, 2012, 2018). Inducers include antiepileptic drugs, rifampin, isoniazid, St. John's wort. Inhibitors include azole antifungals (ketoconazole), non-dihydropyridine calcium channel blockers (diltiazem), macrolide antibiotics, and grapefruit.

To achieve optimal immunosuppression, mTOR inhibitors are used with other immunosuppressants. When CsA and prednisone are used with SRL, the dosage of SRL needs to be closely monitored (Product Information 2018). Sirolimus at a concentration >15 ng/mL is associated with hypertriglyceridemia, thrombocytopenia, and leukopenia, while a concentration <5 ng/mL is associated with a higher likelihood of acute rejection in kidney transplantation. Also, side effects of CsA and SRL have an additive effect when they are used together. For instance, CsA with SRL therapy increases the mean serum creatinine concentration and decreases glomerular filtration rate. Renal function and proteinuria monitoring are needed.

Though mild proteinuria from using the combination of CsA and SRL can be treated with angiotensin converting enzyme inhibitors (ACEI), the addition of ACEI with SRL increases the risk of angioedema. Thus, the combination is only recommended for rescue therapy or to reduce CsA toxicity with everolimus. Tacrolimus with SRL is also associated with decreased renal function. The combination of MMF and SRL is associated with anemia and cast nephropathy.

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Corticosteroids in Immunosuppression

Caroline Marzbani and Arvind Bhimaraj

Contents

Abstract

Corticosteroids have been utilized as mainstay pharmacological intervention for successful organ transplantation since the beginning. Several challenges exist in establishing a balance between achieving a tolerant atmosphere in the host immune system while minimizing the long-term impact of steroids on the body. Corticosteroids are used early in all solid organ transplantation but there is wide variability across various organs and centers in the duration of use and protocols of planned steroid wean. The adverse event profile of steroids is exhaustive and across many organ systems.

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1 Introduction

Corticosteroids are a general term used to describe a group of steroid hormones released by the adrenal cortex and their synthetic analogues. They are further classified into glucocorticoids and mineralocorticoids based on their physiological actions.

The corticosteroids used in transplantation medicine are generally glucocorticoids and used for their immune-modulatory actions on the immune system of the host with an objective to mitigate and minimize rejection. Glucocorticoids are one of the most widely prescribed drugs in the world and the worldwide market for glucocorticoids is estimated to be worth more than USD 10 billion per year (Ramamoorthy and Cidlowski 2016). While steroids alone did not make solid organ transplantation possible, they have been a mainstay background therapy and were the first class of medications that were used to achieve the objective of transplanting between immune non-compatible individuals.

2 Historical Perspective

The discovery of cortisone was centered around treatment of inflammatory disease, especially rheumatoid arthritis, which had debilitating symptoms and consequences prior to such discovery. Philip Hensch and Edward Kendall are credited with the discovery of cortisone and along with the Polish chemist Tadeus Riechtein received the Nobel Prize in Medicine and Physiology in 1950. Philip Hench, as a physician at Mayo Clinic published a series of 30 cases where symptoms of rheumatoid arthritis were relieved with the onset of jaundice, in pregnancy, infection, and surgery (Hench 1938). With a postulation that a "substance X" is secreted naturally in these conditions, and a hunch that it is coming from the adrenal glands, he collaborated with Edward Kendall, a professor of physiology and chemistry who was already studying adrenal hormones. While early work in their collaborative effort and independent work of their Polish competitor Reichten did not succeed in isolation of Cortin, the gloom of World War-II and a supposition of a need of steroid hormones as an anti-stress compound made isolation of and production of Cortin a U.S government priority (Kendall 1971). A culmination of efforts of these physician scientists with involvement of Merck led to the production of Cortisone in 1948. While no specific clinical indication was evident at that time, an insisting patient at Mayo Clinic made Hensch and Kendall try the medication leading to a sensational improvement of symptoms followed by documentation of the anti-inflammatory properties of the substance. As the world witnessed the balance of beneficial and adverse events of cortisone in various scenarios, early work in kidney transplant in

the 1960s revealed, validated, and propagated the ability of steroids to reverse acute rejection in living donor kidney transplant (Goodwin et al. 1962; Starzl and Marchioro 1963). A widespread use of corticosteroids as standard therapy for all kidney transplantation followed.

3 Mechanism of Action (Fig. 1)

Glucocorticoids manifest various immunomodulatory effects through genomic and non-genomic pathways. The genomic mechanism is mediated by binding to the intracellular glucocorticoid receptor (GR) which leads to conformational change in the ligand receptor complex followed by translocation of the complex into the nucleus. In the nucleus, this complex modulates the transcription of specific DNA sequences that lead to inhibition of the synthesis of almost all known inflammatory cytokines by blocking the function of transcription factors, such as nuclear factor-kappa-B (NF-kB) and activator protein-1 (AP-1), two common proinflammatory mediators (Scheinman et al. 1995; Auphan et al. 1995; Rhen and Cidlowski 2005). The GR/Steroid complex also blocks the promoter site of interleukin (IL)-1-alpha and IL-1-beta (Zhang et al. 1997), promoting anti-inflammatory gene transcription of I-kappa-B-alpha, IL-1 receptor-II, lipocortin-1 (annexin 1), IL-10, alpha-2-macroglobulin, and secretory leukocyte-protease inhibitor (Scheinman et al. 1995; Auphan et al. 1995). Glucocorticoids also influence the post translational aspects of proinflammatory mechanisms by diminishing the stability of messenger RNA (mRNA) encoding IL-1, IL-2, IL-6, IL-8, tumor necrosis factor, and granulocyte-macrophage colony-stimulating factor (Tobler et al. 1992).The non-genomic actions of glucocorticoids involve physiochemical interactions with cytosolic GR or membrane-bound GR which unlike genomic effects do not require protein synthesis and occur within seconds to minutes of GR activation (Groeneweg et al. 2012).

Neutrophilia is common while using glucocorticoids as they increase their release from bone marrow and cause a reduction in expression of adhesion molecules on both leukocytes and endothelial cells. This is mediated by a decrease in synthesis and release of prostaglandin mediators of cell adhesion. In contrast, lymphocytes, eosinophils, mast cells, basophils, and dendritic cells decrease in number after administration of glucocorticoids. The total number of natural killer cells remains unchanged. A single dose of cortisol results in a 70% decrease in lymphocytes and a 90% decrease in monocytes, occurring 4–6 h after treatment and persisting for about 24 h. Cell numbers then rise 24–72 h after treatment (Pountain et al. 1993). The decrease in lymphocytes, monocytes, and eosinophils is due to redistribution of these cells rather than cell lysis, although certain types of activated T lymphocytes undergo glucocorticoid-induced apoptosis (Schwartzman and Cidlowski 1994). Glucocorticoids also reduce migration of monocytes and macrophages resulting in decreased tissue accumulation and slight increase in the blood level of these cells. The effect of steroids on monocyte and macrophage functions is variable. Macrophage phagocytosis and clearance of opsonized bacteria by the reticuloendothelial cells are diminished (Atkinson and Frank 1974). Expression of major

promoter of the gene.

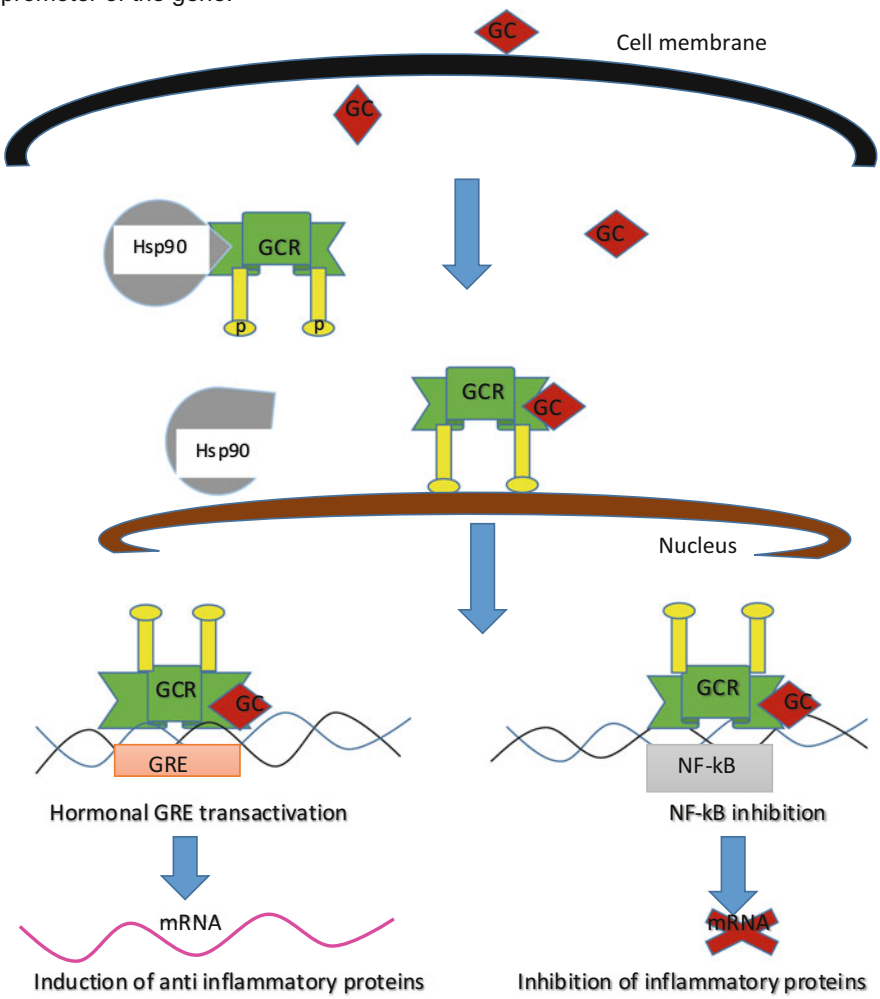

Fig. 1 Pictorial representation of mechanisms of action of glucocorticoids (GC). The GC (Red diamond shape) transverses the cell membrane to bind to the cytoplasmic GC receptor (GCR). This interaction frees the hsp-90 which is usually bound to GCR. The GC/GCR complex then is able to be transported into the nucleus where it impacts the transcription of various proteins through NFk-B inhibition and direct activation of the transcription of anti-inflammatory mRNA via glucocorticoid response element (GRE) which is a short sequence of DNA within the promoter of the gene

histocompatibility complex class I (MHC-I) and chemokine secretions are not affected or may in fact be increased in the presence of glucocorticoids. In contrast, the expression of MHC class II and antigen presenting function are reduced (Gerrard et al. 1984). The circulatory levels of B and T lymphocytes are reduced by glucocorticoids mainly because of redistribution of these cells to the

reticuloendothelial tissues and this effect is more pronounced on T cells than B cells. High doses of glucocorticoids inhibit immunoglobulin synthesis (Grayson et al. 1981) and decrease production of components of the complement system (Caren and Rosenberg 1966).

4 Types of Glucocorticoids and Dose

Glucocorticoids share the similar anti-inflammaory action and side effects. They differ in potency, duration, and mineralocorticoid activity. Prednisone is the most commonly used steroid. It is a prodrug and requires first-pass metabolism in order to be tranformed to the active metabolite, prednisolone. In patients with severe liver dysfunction prednisolone is preferred. Methylprednisolone differs in only a methyl group. Table 1 lists dose equivalents of the various glucocorticoids. Generally, in the context of solid organ transplant, for oral prednisolone, a low dose is considered up to 7.5 mg/d, medium dose >7.5 mg but <30 mg/d and high dose >30 mg but <100 mg/d, and very high dose is considered >100 mg/d. A pulse of methylprednisolone is considered between 250 and 1,000 mg/d for 1–3 days (Buttgereit et al. 2002).

5 Clinical Indications for Use in Solid Organ Transplant

5.1 Intraoperative Steroids

As an induction agent post solid organ transplant, steroids are used alone or along with other immunomodulatory agents intra-operation or immediately post-operation to initiate the process of adaptation or immunologic tolerance to the allograft. While

Table 1 Glucocorticoid comparisons and dose equivalents for glucocorticoid potency (Schimmer and Funder 2011)

Type of steroid	Dose equivalent for glucocorticoid potency	Mineralocorticoid potency	Half life
Cortisone	25	0.8	8–12 h
Hydrocortisone	20	1	8–12 h
Fludrocortisone	N/A	125	8–12 h
Prednisolone	5	0.6	18–36 h
Prednisone	5	0.6	18–36 h
Methylprednisolone	4	0.6	18–36 h
Dexamethasone	0.75	0	36–72 h

Table 2 Dose of intraoperative methylprednisolone at the time of solid organ transplant for different organs

Induction with methylprednisolone	Dose
Heart	1 g
Lung	1–1.5 g
Kidney and pancreas	200–500 mg
Liver	500 mg

steroids have been the mainstay of rejection prevention medications from the early days of transplantation, other potent induction agents like Thymoglobulin and IL-2 inhibitors have made steroids an adjunct treatment more than a primary induction strategy. The appropriate dose of intraoperative methylprednisolone has not been studied and various centers and organ programs use varying doses. Table 2 lists the dose of intraoperative methylprednisolone at our center. Typically, this intraoperative dose is followed by a gradual taper down to the maintenance dose over days to weeks depending on the dose started at and gradations of taper. Protocols range from starting at a high oral dose with a gradual long taper to others with an early transition to intravenous dosing followed by a higher decrement and faster wean down to maintenance dose. There are no studies comparing the various down titration regimens.

5.2 Maintenance Dosing and Steroid Withdrawal

The dose of steroids used to maintain immunological quiescence has decreased significantly from the advent of solid organ transplant: Most programs reach a maintenance dose of 5–10 mg of oral prednisone which is sometimes withdrawn completely after 6 months to 1 year after transplantation. While there has been a push towards steroid taper and wean in the context of minimizing long-term side effects of these medications, it is not clear if such a strategy makes a difference in the long-term outcomes for the graft or the patients across all organ groups. Also, it is not clear if the removal of the low dose of prednisone used in the current era mitigates the presumed side effects of prednisone in all patients while not increasing the risk on graft survival. While some centers tailor prednisone wean for those at lower immunological risk of rejection, some centers, more so in thoracic transplants, use steroids at the low maintenance dose for a lifetime (especially in lung transplants where rejection burden is high). While safety of steroid withdrawal has been established in many studies in all organs (Baran et al. 2011; Luan et al. 2009) especially in low rejection risk patients, the generalizability of such findings in individuals at a higher risk of rejection and a possibility of an unexpected trigger (like an infectious trigger) inciting acute rejection after achieving the so-called tolerant state off of steroids (Wang et al. 2010) have made universal adaptation of such strategy difficult. The strategy to withdraw maintenance steroids is also

confounded by the fact that clinical studies have varied in the timing of withdrawal and concomitant immunosuppressant medications. Despite all these controversies there seems to be some general principles that govern this decision: (1) Most low risk profile patients can be safely weaned off corticosteroids; (2) Steroid withdrawal with a background therapy of tacrolimus is considered to be safer than cyclosporine or only an mTOR inhibitor; (3) Early weaning is recommended: while steroid weaning is done within weeks in kidney transplants most thoracic programs do so in 6 months to 1 year; (4) While surveillance studies have focused on cellular rejection, recent acceptance and surveillance methods for antibody mediated rejection have left unanswered questions of the risk of AMR during the wean and careful surveillance is recommended. In our experience, withdrawal of steroids in patients who have been on prednisone for years seems to predispose them to AMR than ACR. The most commonly used tapering regimen includes (Saag and Furst 2019).

- 5–10 mg/day every 1–2 weeks from an initial dose above 40 mg of prednisone or equivalent per day.
- 5 mg/day every 1–2 weeks at prednisone doses between 40 and 20 mg/day.
- 2.5 mg/day every 2–3 weeks at prednisone doses between 20 and 10 mg/day.
- 1 mg/day every 2–4 weeks at prednisone doses between 10 and 5 mg/day.
- 0.5 mg/day every 2–4 weeks at prednisone doses from 5 mg/day down. This can be achieved by alternating daily doses, e.g., 5 mg on day one and 4 mg on day two.

5.3 Treatment in Acute Rejections

Methylprednisolone is the mainstay of treatment for acute cellular rejection (ACR) and has been adapted for basic therapy for antibody mediated rejection (AMR). ACR without overt organ derangement can sometimes be managed with increasing the dose of oral prednisone (in our institution, pathological 2R ACR in heart transplant with no graft dysfunction or hemodynamic derangements is treated with 100 mg prednisone daily for 3 days followed by a taper) while any suggestion of organ dysfunction is treated with an administration of intravenous pulse steroids. AMR is usually treated with high dose steroids with 500–1,000 mg methylprednisolone for 3–5 days while other strategies of antibody removal and B-cell suppression are being implemented.

6 Side Effects (Saag and Furst 2019)

Chronic steroid use has many physiological implications involving various organ systems. Many transplant patients a have other risk factors and are taking medications which can compound such effects of steroids. Table 3 lists the impact

Table 3 Impact of steroids on various organ systems of the human body that contributes to adverse effects in the setting of chronic long-term use

Organ system	Adverse effects
Skin	Skin atrophy, impaired wound healing, acne
General	Cushingoid appearance, weight gain
Eyes	Cataracts, glaucoma, exophthalmos, central serous chorioretinopathy
Cardiovascular	Fluid retention, hypertension, dyslipidemia, premature atherosclerotic disease, atrial fibrillation, atrial flutter
Nervous system	Stroke, pseudotumor cerebri, akathisia, psychosis, panic disorder, memory impairment, insomnia
Gastrointestinal	Gastritis, fatty liver, visceral perforation, pancreatitis
Bone and muscle	Osteoporosis, osteonecrosis, proximal myopathy
Endocrine	Hyperglycemia, secondary adrenal insufficiency
Infectious	Increased risk of bacterial, viral, and fungal infection

of glucocorticoids on various organ systems. Low doses of glucocorticoids (e.g., prednisone <5 mg/day) are associated with fewer adverse effects (Pincus et al. 2011), hence efforts are made to reduce dosing on the long term. Most glucocorticoid toxicity is at least partially reversible over time with early dose reduction (or withdrawal), detection, and treatment of contributing co-factors (Saag and Furst 2019). Skin ecchymosis and purpura often affect the sun-exposed areas of the dorsum of the hand and forearm. Acne, skin atrophy, impaired wound healing are common. Cataracts are common even with lower doses of <5 mg/day and is typically bilateral with posterior subcapsular involvement. Studies in non-organ transplant population using chronic steroids have shown an increase in adverse cardiovascular outcomes including fluid retention (e.g. glucocorticoids with mineralocorticoid property), hypertension, increased risk of premature atherosclerotic disease, stroke, heart failure, atrial fibrillation, atrial flutter, dyslipidemia and all-cause mortality. Cardiovascular disease risk is dose-dependent (Wei et al. 2004). It is unclear if this is true in the transplant setting due to inability to do a well-designed study to eliminate the influence of confounders. Glucocorticoid-induced reduction in ACTH release contributes to dyslipidemia by downregulating LDL receptors (Berg and Nilsson-Ehle 1996). Gastrointestinal effects can include peptic ulcer disease, gastritis, fatty liver, visceral perforation, and pancreatitis. The combination of glucocorticoids and NSAIDs results in a synergistic increase in the incidence of gastrointestinal events by two to fourfold increase. American College of Rheumatology (ACR) Task Force osteoporosis guidelines suggest that all patients taking glucocorticoids (any dose with an anticipated duration of ≥3 months) should maintain a total calcium intake of 1,000 to 1,200 mg/day and vitamin D intake of 600 to 800 international units/day through either diet and/or supplements to avoid osteonecrosis. Most studies have found that the risk of osteoporosis is low (<3%) in

patients treated with doses of prednisone <15–20 mg/day (Jones and Mont 2019). If patients are on long-term steroids most centers screen on regular intervals with DEXA scan. Myopathy is uncommon and typically presents with painless proximal motor weakness in both the upper and lower extremities. Other confounding medications like statins also need to be reviewed as culprit medications for myopathy. Mood disorders and emotional lability are more common in patients with a family history of depression or alcoholism. Psychosis occurs at high doses of prednisone usually above 20 mg/day and for prolonged periods while akathisia can occur even at low doses. Hyperglycemia is probably the most common and obvious impact of systemic glucocorticoids causing a dose-dependent increase in the level of serum blood glucose but the development of de novo diabetes in a patient with initially normal glucose tolerance is uncommon (Olefsky and Kimmerling 1976a). Risk factors for new-onset hyperglycemia during glucocorticoid therapy are thought to be the same as those for other patients, including a family history of diabetes, increased age, obesity, and a history of gestational diabetes (Olefsky and Kimmerling 1976b). Secondary adrenal insufficiency due to long-term use of glucocorticoids is more likely to develop due to suppression of the hypothalamic-pituitary-adrenal (HPA) axis in those who receive high doses (>20–30 mg prednisolone or equivalent) of systemic GCs for >3 weeks, those who develop Cushingoid features (Saag and Furst 2019) and those who have received an evening/bedtime dose of ≥5 mg of prednisone for many weeks. These patients should be treated like any patient with secondary adrenal insufficiency and if weaning of steroids is indicated should undergo tapering regimens. If these patients undergo minor stress while being on glucocorticoid treatment, they might require dose increase by double for 1–2 days and for severe stress three to tenfold dosage increase for 2–3 days. If steroids are used for <2–3 weeks, the recovery is expected and hence considered low risk for HPA suppression. Steroids can be stopped without taper in this scenario. In patients with intermediate risk using tapering regimens or HPA function test in certain case scenarios (e.g. planned elective surgery) could be beneficial (Table 4).

Table 4 Mineralocorticoid stress dose steroid dosing to be considered in situations of physiological stress

Stress (medical/surgical)	Steroid stress dose	Taper after the stress resolves
Minor, e.g., mild febrile illness, colonoscopy, <1 h anesthesia	25 mg or 30–50 mg/m^2 IV or PO hydrocortisone or equivalent	None
Moderate, e.g., pneumonia, multiple tooth extraction	50 mg or 50–75 mg/m^2 IV hydrocortisone or equivalent	Taper over 1–2 days
Major, e.g., severe burn, sepsis, major surgery	100 mg or 100 mg/m^2 IV hydrocortisone or equivalent	Taper over 1–3 days

7 Interactions of Glucocorticoids with Other Drugs (Liapi and Chrousos 1992)

Glucocorticoids can cause severe hypokalemia once given with other drugs such as Amphotericin B, diuretics, and can increase Digoxin toxicity. Glucocorticoids can decrease the blood level of some of the medications such as aspirin, warfarin, insulin, oral hypoglycemic agents and increase the blood level of cyclophosphamide or cyclosporine (Liapi and Chrousos 1992). Plasma levels of glucocorticoids can be decreased by use of antacids, cholestyramine or increased by cyclosporine, itraconazole, oral contraceptives. (Liapi and Chrousos 1992; Foisy et al. 2008; Saberi et al. 2013) Due to lack of a commercially available serum level for corticosteroids, it is not practical to adjust dosing despite potential pharmacokinetic interactions.

8 Conclusion

Glucocorticoids remain to be widely used in organ transplantation from organ induction to maintenance of immune quiescence, and treatment of acute rejection episodes. While long-term exposure can cause many deleterious effects, they remain a very useful armamentarium in post-transplant setting. Future studies need to focus on alternate delivery mechanisms that can have more targeted impact on the immune system while avoiding systemic side effects. Also, efforts to minimize dose-duration exposure to steroids need to be consolidated for better consensus. It is important to understand this class of drugs in the context of their role, pharmacokinetic, pharmacodynamics, adverse effects and clinical applications in order to utilize them appropriately to maintain transplanted organ vitality while preserving the rest of the body from the long-term impact of these medications.

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Induction Therapy and Therapeutic Antibodies

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Abstract

Prevention of allograft rejection is one of the crucial goals in solid organ transplantation to ensure durability of the graft and is chiefly mediated by cellular and humoral pathways targeting cell surface alloantigens. The risk of rejection is highest in the first post-transplant year and wanes with time albeit the risk always exists and varies with the type of organ transplanted. Induction therapies refer to the use of high-intensity immunosuppression in the immediate post-operative period to mitigate the highest risk of rejection. This term encompasses chiefly the use of antibody therapies directed against one of the key pathways in T-cell activation or abrogating effects of circulating alloantibodies. These antibodies carry more potent immunomodulatory effect than maintenance immunosuppressive therapy alone and many of them lead to durable immune cell depletion. A variety of monoclonal and polyclonal antibodies have been utilized for use not only for induction therapy, but also for treatment of allograft rejection when it occurs and as components of desensitization therapy before and after transplantation to modulate circulating alloantibodies.

Keywords

Desensitization therapies · Induction immunosuppression · Rejection treatment · Solid organ transplantation

1 Introduction

Organ transplantation is one of the most remarkable medical achievements of the twentieth century, leading to the application of this life-saving procedure as the definitive therapy for many types of end-organ failure. Joseph E Murray performed the first successful renal transplant between identical twins in 1954 in Boston, followed by the first lung transplant by Dr. James Hardy in 1963, the first successful liver transplant by Dr. Thomas E. Starzl in 1967, the first heart transplant by Christian Barnard in 1967, and the first successful bone marrow transplant by E. Donnall Thomas in 1968 (Enderby and Keller 2015). Prevention of allograft rejection is one of the crucial goals in solid organ transplantation, ensuring the durability of the allograft. The ideal form of immunosuppression attains an equilibrium between inducing donor-specific tolerance, on the one hand, and preserving sufficient function of the immune defenses that protects the host from the dangers of infection and cancers, among other. The risk of rejection is highest in the first post-transplant year and wanes with time albeit the risk always exists. Furthermore, the rejection risk varies by types of organ transplanted. For example, the liver is considered an "immune-privileged organ" with accompanying lower rejection probability compared to many other solid organs. In fact, studies have shown that 20–60% of liver transplant recipients are able to achieve liver graft immune tolerance after immunosuppression withdrawal.

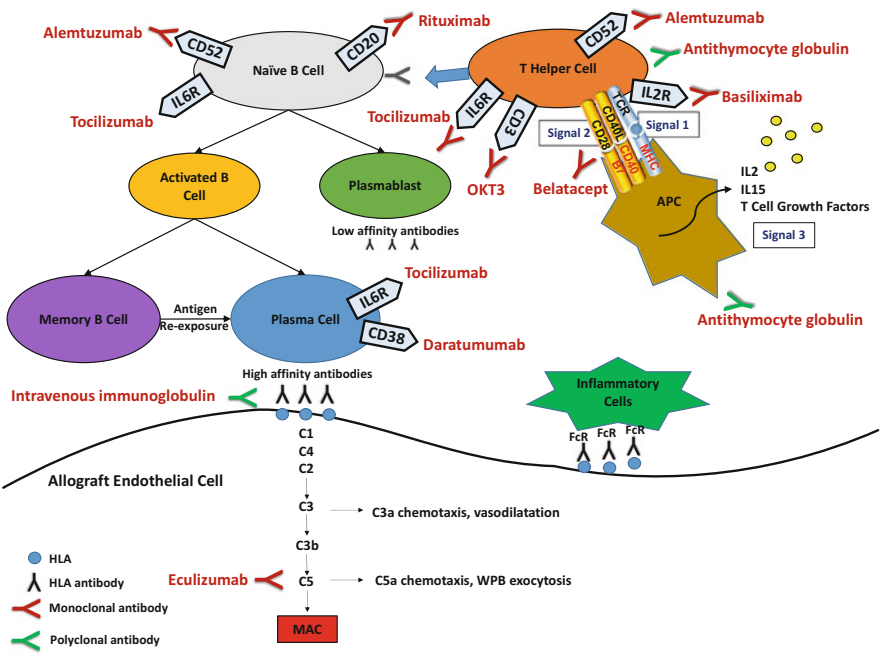

Fig. 1 T-cell and B-cell activation in allograft injury. T-cell activation is described by the 3-signal model. Signal 1 involves the T-cell receptor engagement of an antigen-MHC tandem. In addition to the MHC-allopeptide complex, a costimulatory signal, signal 2, is required for T-cell activation. T cells require cytokine signals to propagate a cascade of pro-survival and proliferation signals (Signal 3). Humoral response and antibody-mediated injury. The humoral response involves T-cell–dependent B-cell activation and formation of either memory B cells or high-affinity antibody-secreting plasma cells. Upon re-exposure to antigen, memory B cells proliferate and differentiate into plasma cells. Binding of donor-specific HLA antibody to the allograft endothelium causes injury through complement-dependent and-independent mechanisms. HLA antibody-antigen complexes trigger complement cascade activation and formation of the MAC, which causes endothelial-cell lysis and destruction. Complement factors are also chemotactic and cause vasodilation, mast-cell release. HLA antibody binding may directly recruit inflammatory cells via FcR adhesion. Desensitization treatments are shown in red with corresponding targets. FcR Fcreceptor, MAC membrane attack complex

The three arms of immunosuppression therapies are induction, maintenance, and treatment of rejection. Understanding the key mechanistic regulators involved in allograft rejection allows the design of therapies that intercept these deleterious for the allograft pathways (Fig. 1).

In evolutionary terms, the immune system has developed into a complex organization of cellular, antibody, and cytokine responses designed to protect the host against foreign pathogens while preventing injury to self. In transplantation, activation of these systems leads to injury and rejection of the allograft. Our understanding of the immune response has led to development of effective immunosuppressive

regimens that have made solid organ transplantation possible and increasingly provided more effective tools to treat rejection when it occurs.

One such main target of suppressive pharmacotherapies is inhibiting T-cell activation and proliferation (Nankivell and Alexander 2010). This pathway is the key executioner of the immune system that ultimately leads to cell death and graft dysfunction. Mitchison was among the first to describe the cell-mediated features of allograft rejection. Immune responses against the donor organs also involve other mechanisms including humoral (antibody-mediated) as well as those mediated by other cell types. The antigens responsible for T-cell activation and subsequent rejection of genetically disparate tissues are of the major histocompatibility complex (MHC), which have evolved unprecedented genetic diversity (Halloran 2004). They are encoded by over 40 loci on the short arm of chromosome 6 and in humans, they are called human leukocyte antigens (HLA). The role of MHCs is to surveille their environment and present antigenic peptides to T cells. They are divided into two classes – Class I molecules are expressed on all nucleated cells and present peptides derived from intracellular sources (e.g., intracellular viruses, self-antigens, tumor antigens) to CD8 cells. Class II molecules are present only on antigen-presenting cells (APCs) such as dendritic cells, activated macrophages, and endothelial cells as well as B cells. The latter present extracellular antigens to CD4 cells (Iwasaki and Medzhitov 2010; Smyth et al. 2006). However, non-HLA antigens are also increasingly recognized to be involved in some forms of rejection.

T-cell activation is described by the 3-signal model (Ingulli 2010) (Fig. 1). Signal 1 involves the T-cell receptor engagement of an antigen-MHC tandem. As the allograft undergoes substantial ischemic injury during the process of donor death, organ procurement, and operative procedure, there is heightened presentation of donor antigens by passenger APCs (Chong and Alegre 2012). The host T cells recognize these complexes as foreign and trigger direct cytotoxic T-cell activation (Moreau et al. 2013). Alternatively, as an indirect mechanism host APCs surveille the milieu and present donor alloantigens coupled with recipient MHC class II molecules, stimulating helper CD4+ T cells (Smyth et al. 2006; Moreau et al. 2013). This pathway is thought to be more pertinent to the setting of chronic and late acute rejection as well as coronary allograft vasculopathy (Moreau et al. 2013). In human studies, onset of acute rejection in heart transplant recipients appears to be triggered by T-cell responses to a single dominant epitope on one alloantigen. However, in the setting of recurrent or chronic rejection, T-cell reactivity could be directed to other epitopes within the MHC molecule or other alloantigens on the graft. This process is called antigen spreading (Vanderlugt and Miller 2002).

In addition to the MHC-allopeptide complex, a costimulatory signal, signal 2, is required for T-cell activation (Brook et al. 2006). In fact, T cells become anergic when presented with signal-1 alone without costimulation. Costimulatory pathways include those mediated by the CD28 T-cell receptor binding to B7-1 (CD80) or B7-2 (CD86) on APCs (Brook et al. 2006). CD28 is constitutively expressed on 95% of CD4+ T cells and 50% of CD8+ T cells in humans (Smith et al. 2012). As a checkpoint inhibitor of T-cells also express costimulatory antigens which serve as checkpoint inhibitors of cell activation. One of the best characterized ones involves

the T-lymphocyte-associated antigen 4 (CTLA4), a homologue of the CD28 molecule, which is induced on activated T cells. CTLA4 also binds to B7-1 and B7-2 and inhibits IL-2 production and cell cycle progression (Moreau et al. 2013).

Finally, it has been well recognized that T cells require cytokine signals (signal 3) to propagate a cascade of pro-survival and proliferation signals (Moreau et al. 2013; Mosser and Edwards 2008). In fact, during T-cell activation the calcineurin pathways is activated, leading to dephosphorylation of the cytoplasmic nuclear factor of activated T cells (NFAT), permitting its translocation to the nucleus, where it binds to the IL-2 promoter (Moreau et al. 2013). IL-2 expression is a key stimulator of T-cell proliferation and activation.

2 Induction Therapies

Induction therapies refer to the use of high-intensity immunosuppression in the immediate post-operative period to mitigate the highest risk of rejection. This term encompasses chiefly the use of antibody therapies directed against one of the key pathways in T-cell activation, described above. These antibodies carry more potent immunomodulatory effect than maintenance immunosuppressive therapy alone and many of them lead to durable immune cell depletion. The employment of these various induction strategies should be guided by the individual patient risk profile, weighing in the immune priming of the recipient as well as his risk of infection or experiencing other adverse effects of the therapy employed. However, despite our growing knowledge of the risk factors for rejection in the various solid organ transplant types, there is a marked lack of integrated risk stratification tools to help guide the use of induction therapies with their competing risks. Defining a model that incorporates the calculus of the individual's risk of rejection and infection would aid with the challenging task of personalizing immunosuppression and furthermore help to improve the design of clinical trials in transplantation (Cippa et al. 2015).

The use of antibody induction therapies is highly heterogeneous across solid organ types. According to the Organ Procurement and Transplantation Network and the Scientific Registry of Transplant Recipients (SRTR) Annual Data Report from 2019 (SRTR 2019), antibody induction therapy was employed in 91.9% of kidney (Hart et al. 2021), 90% of pancreas (Kandaswamy et al. 2021), 28% of liver (Kwong et al. 2021), 74.1% of intestinal (Horslen et al. 2021), 49% of heart (Colvin et al. 2021), and 78% of lung (Valapour et al. 2021) transplant recipients.

Below we describe the various induction regimen employed historically and in modern clinical practice with their mechanistic underpinnings and important side effect profile.

2.1 Antibodies (Table 1)

2.1.1 Muromunab-OKT3

Antibody therapies have entered clinical practice since the 1980s. They include T-cell-depleting and nondepleting agents and can be monoclonal or polyclonal

Table 1 Antibody preparations utilized in solid organ transplantation

Drug	Mechanism of action	Approved indication	Dose	Administration	Infusion monitoring	Protocol	Premedication	Contra-indication	Toxicities
IVIg	– Neutralization of circulating antibodies – different effects on B-cell activation and maturation	– Primary humoral immunodeficiency – Immune thrombocytopenic purpura –	1–2 g/kg IV (max 80 kg)	Low rate IV, 4–6 h	Standard monitoring including heart rate and blood pressure (more closely at the beginning of the infusion)	Total dose spread over 2–4 days Discuss repeating infusions every 4 weeks	Preventive anticoagulation therapy	–	Infusion reactions, renal impairment, thrombosis and transfusion-related acute lung injury
ATG	Polyclonal depletion of T (and B) cells via ADCC and CDC	– Prpohylaxis and treatment of acute rejection in kidney transplant	1.5 mg/kg/d IVx4–14d		Standard monitoring including heart rate and blood pressure (more closely at the beginning of the infusion)		Antihistamine, acetaminophen and methylprednisolone 100 mg IV 30 min before infusion		Urinary tract infection, abdominal pain, hypertension, nausea, shortness of breath, fever, headache, anxiety, chills, increased potassium, low counts of platelets and white blood cells
Alemtuzumab	Humanized rat monoclonal anti-CD52 antibody present on lymphocytes, natural killer (NK) cells and macrophages	Treatment of B-cell chronic lymphocytic leukemia	30 mg IVx1	IV infusion over 4 h	Standard monitoring including heart rate and blood pressure (more closely at the beginning of the infusion)		Antihistamine, acetaminophen, and methylprednisolone before infusion		Infection, profound leukopenia
Basiliximab	Non-depleting anti-CD25 monoclonal antibody (IL2R antagonist)	Prophylaxis of acute organ rejection in patients receiving renal transplantation	20 mg IV day 0 and day 4 post transplant	IV bolus or diluted	Standard monitoring including heart rate and blood pressure	Infuse over 20–30 min	None	Risk of anaphylaxis with history of prior exposure to basiliximab	Infection, pain, nausea, peripheral edema, hypertension, anemia, headache, hyperkalemia, hypercholesterolemia, increase in serum creatinine, hypophosphatemia

Rituximab	CD20+ B cells depletion (CDC + ADCC)	– Non-Hodgkin's lymphoma – Chronic lymphocytic leukemia – Autoimmune disorders	Usually 375 mg/m^2 IV	IV Begin with low rate 50 to 100 mg/h and increase in the absence of infusion toxicity	Careful monitoring, risk of severe reaction and tumor lysis syndrome	1 to 4 infusions 1 week between each infusion	Antihistamine, acetaminophen, and methylprednisolone 100 mg IV 30 min before infusion	–	Infusion-related reaction, mucocutaneous reaction, HBV reactivation, progressive multifocal leukoencephalopathy
Tocilizumab	Humanized monoclonal against soluble and membrane-bound Interleukin-6 receptor (IL-6R)	Rheumatoid arthritis, giant cell arteritis, polyarticular and systemic juvenile idiopathic arthritis, cytokine release syndrome, rapid respiratory decompensation due to COVID-19	8 mg/kg monthly, maximal dose 800 mg, for 6 mo	IV over 1 h	Standard monitoring	1–6 infusions monthly			Tuberculosis, bacterial, invasive fungal, viral, and other opportunistic infections. Hepatic dysfunction, neutropenia, and thrombocytopenia
Eculizumab	Recombinant humanized monoclonal antibody – complement C5 inhibitor	– PNH – aHUS – Generalized myasthenia gravis – Neuromyelitis optica spectrum disorder	900–1,200 mg IV	IV infusion over 35 min	Standard monitoring	– 1,200 mg at release of cross-clamp – 900 mg on day 1, then weekly for 3 weeks – 1,200 mg at weeks 4, 6 and 8	Vaccinate patients against N. meningitidis at least 2 weeks before the use eculizumab	Patients not vaccinated against N. meningitidis, unless the risks of delaying treatment outweigh the risks of meningococcal infection	Increased risk of Neisseria meningitidis infections, upper respiratory tract infection

(continued)

Table 1 (continued)

Drug	Mechanism of action	Approved indication	Dose	Administration	Infusion monitoring	Protocol	Premedication	Contra-indication	Toxicities
Belatacept	Humanized anti-CD28 (CTLA-4) antibody	Prophylaxis of organ rejection in adult patients receiving a kidney transplant	10 mg/kg IV initial, 5 mg/kg maintenance monthly	IV infusion over 30 min	Standard monitoring	10 mg/kg IV day 1, 5, week 2, 4 then 5 mg/kg monthly		EBV seroegative or unknown EBV status Liver transplant	PTLD, progressive multifocal leukoencephalopathy, other infections, allograft rejection
Daratumumab	Fully human IgG1-kappa monoclonal antibody against CD38 on plasma cells	Multiple myeloma	Not established				Corticosteroids, antipyretics and antihistamines prior to infusion		Infusion reaction, neutropenia, thrombocytopenia

preparations. The first monoclonal antibody to be approved for clinical use in humans was OKT3, which is a T-cell depleting therapy (Post et al. 2005). OKT3 is a murine monoclonal IgG2a antibody that specifically reacts with the T-cell receptor-CD3 complex on the surface of circulating human T cells. It blocks T-cell proliferation and differentiation. Adverse effects were relatively common including development of cytokine release syndrome owing to its propensity to initially activate T cells, releasing TNF-a and IL-2, aseptic meningitis, intragraft thrombosis, seizures, pulmonary edema, and increased risk of post-transplant lymphoproliferative disease and infection related to overall augmented immunosuppression. Furthermore, as a non-human antibody, it elicits neutralizing anti-antibody response in 44–89% of patients who received it, depending on the population treated (Kimball et al. 1995). As such OKT3 is no longer used in clinical practice and has been replaced by chimeric and humanized monoclonal antibodies that allow compatibility for human use.

2.1.2 Antithymocyte Globulins (ATG)

Polyclonal T-cell depleting therapies with antithymocyte globulins (ATG) have emerged as potent immunomodulators used both as upfront induction therapies or for treatment of acute rejection (Bonnefoy-Berard et al. 1991). These preparations are purified immunoglobulins derived from horses (hATG) or rabbits (rATG) immunized with human thymocytes and contain antibodies against diverse antigen-combining sites and epitopes (Bonnefoy-Berard et al. 1991). RATG has supplanted hATG in clinical use due to its superior potency and tolerability.

Despite limited evidence from randomized clinical trials, ATGs have been widely used as induction agents in solid organ transplantation. ATG induces predominantly T lymphocyte depletion through antibody dependent cell cytotoxicity (ADCC), complement-dependent cytotoxicity (CDC), opsonization and activation of programmed cell death pathway via antibody-induced and cytokine mediated upregulation of CD178. ATG also attenuates T-cell activation by downregulation of the T-cell receptor/CD3 complex, CD2, CD4, CD5, CD6, and CD8 (Preville et al. 2001). Peripheral T-cell depletion appears to be targeted at naïve cells, relatively sparing memory and regulatory T cells (Ruzek et al. 2009). About half of the patients treated with rATG recover more than 50% of initial lymphocyte count at 3 months but reconstitution is highly variable and prolonged lymphocyte depletion has been observed in some patients.

ATG also appears to control B-cell pathways by reducing Th populations, including B-cell complement-mediated lysis by binding to cell surface proteins shared by B and T cells, and by binding to unique B-cell surface marker, resulting in interference with B-cell activation and induction of apoptosis. ATG also impairs thymopoiesis, thus leading to durable lymphopenia with >90% reduction in circulating T cells (Bonnefoy-Berard et al. 1991). Its effects last approximately 3 months for most patients, and in some, for over 1 year post administration.

Due to initial T-cell activation upon surface receptor binding, ATG can also cause cytokine release syndrome (Bonnefoy-Berard et al. 1991). In milder cases it presents as high-grade fever, chills, and possibly rigors during or shortly after infusion. In

severe cases pulmonary edema with cardio-respiratory depression and even death may occur (Enderby and Keller 2015). To minimize such reactions, it is administered as a slow infusion over 6–8 h with premedication with antipyretics, antihistamines, and corticosteroids. Serum sickness can occur in 5–10% of patients with symptoms of fever, rash, arthralgias, and myalgias, occurring 5–15 days post ATG therapy. Thrombocytopenia and leukopenia are one of its most frequent side effects, experienced by 14–30% of patients (Enderby and Keller 2015). Severe and prolonged lymphocytopenia may last over 1 year. Due to the significant lymphopenia, ATG's most serious complications include the heightened risks of infection and malignancy (Enderby and Keller 2015). Hence, prophylaxis against CMV and PCP are obligatory peri-ATG administration. Malignancies such as lymphoma and post-transplant lymphoproliferative disorder (PTLD) are much more common with ATG than IL-2 blocking therapies (discussed below). Interestingly, in a retrospective registry analysis, polyclonal ATG use in renal transplantation was associated with higher cardiovascular mortality (Meier-Kriesche et al. 2002). Prolonged CD4 T lymphocyte depletion has been associated with progression of atherosclerosis and cardiovascular mortality (Ducloux et al. 2010).

2.1.3 IL-2 Blockade

In the 1990s, two nondepleting monoclonal anti-CD25 antibodies directed against the IL-2 receptor were introduced: basiliximab and daclizumab (production of the latter was later discontinued). As described above, IL-2 is a major growth factor for activated T lymphocytes. Moreover, T-cell proliferation is a central event leading to graft rejection. IL-2 receptor antagonists bind the Tac-chain component of the IL-2 receptor, which is expressed in only a small fraction of activated lymphocytes (Enderby and Keller 2015). Hence, anti-IL2R therapies can potentially provide a more targeted immunomodulation compared to its polyclonal T-cell depleting counterparts. Additionally, through DNA recombinant technology most of the murine portion of the antibody was replaced with human acid sequences (creating chimeric antibody in the case of basiliximab, or humanized antibody in the case of daclizumab), thus circumventing the problems with antigenicity and short half-life that presented a challenge with OKT-3 therapies (Enderby and Keller 2015).

Basiliximab is well tolerated with no significant clinical toxicities or cytokine release syndrome. In fact, in clinical trials rates of adverse effects observed in basiliximab treated patients have been comparable to those in the placebo group (Kahan et al. 1999). Conveniently, basiliximab has no significant interactions with other immunosuppressive therapies, is not associated with increased risks of infections or malignancy, and leads to stable blood counts with no associated cell depletion. Due to its safety profile, it has become the induction agent of choice in lower immunological risk SOT recipients. Adverse effects are generally mild and include infections, pain, nausea, peripheral edema, hypertension, anemia, headache, hyperkalemia, hypercholesterolemia, increase in serum creatinine, and hypophosphatemia.

2.1.4 Outcomes on IL2RA vs ATG Induction Regimen

IL2RA and ATG are the most commonly used induction regimen in all SOT. There is now growing evidence, largely based on observational studies, on their comparative effectiveness and inherent limitations.

Kidney Transplantation

The kidney transplant literature is leading the way in providing insights on the comparative effectiveness of various induction therapies. In the late 1990s–early 2000s, a notable meta-analysis of randomized trials compared induction strategy with IL2RA, ATG, and placebo in a low immunological risk cohort and demonstrated IL2RA leads to 1/3 lower rates of biopsy-proven rejection and graft loss at 1 year compared to placebo (Webster et al. 2010). ATG was not superior to IL2RA in this analysis but IL2RA had better safety profile. This study formed the basis for 2009 Kidney Disease Improving Global Outcomes (KDIGO) guidelines for management of the kidney transplant recipients which advocated for the routine use of induction therapy with IL2RA as first-line therapy in all kidney transplant recipients (grade 1B) (Kidney Disease: Improving Global Outcomes Transplant Work Group 2009). KDIGO recommended that lymphocyte-depleting agents be reserved for patients at high immunological risk (grade 2B) defined as high number of HLA mismatches, younger recipient age, older donor age, black ethnicity (in the USA), panel reactive antibodies >0%, presence of a donor-specific antibody, blood group incompatibility, delayed onset of graft function and cold ischemia time >24 h (Hellemans et al. 2017).

However, the studies that formed the basis for this recommendation were primarily based on pre-tacrolimus maintenance regimen no longer relevant to contemporaneous clinical practice. Since the implementation of the triple regimen tacrolimus, mycophenolate mofetil and corticosteroids as the standard maintenance immunosuppression therapy, there has been a significant reduction in 1-year rejection rates form ~50% in the 1990s to ~10–15% in the current era (Hellemans et al. 2017). In that context, analyses based on more recent data have repeatedly shown no clinically significant differences in graft survival in standard risk patients when IL2RA is used vs control (Gralla and Wiseman 2010). Some of the studies report statistically significant reduction in acute rejection rates of 1–4% without impact on long-term graft outcomes (Lim et al. 2010; Tanriover et al. 2015).

On the other hand, antibody induction therapy plays a clearly defined role in higher immunological risk patients. The evidence stems from two randomized controlled trials comparing IL2RA vs rATG induction. In the first trial, maintenance therapy consisted of CsA, mycophenolate mofetil, and steroids (Brennan et al. 2006). In that study, rATG induction reduced the rates and severity of rejection by nearly half both at 1- and 5-year follow-up (Brennan et al. 2006; Brennan and Schnitzler 2008). In the second study, background maintenance therapy reflected modern practices and consisted of tacrolimus, mycophenolate mofetil, and corticosteroids (Noel et al. 2009). The study also showed significantly lower incidence and severity of rejection with rATG vs IL2RA by nearly half both at 1- and 5-year follow-up (Brennan et al. 2006; Brennan and Schnitzler 2008). Apart from the

clear benefit of ATG over IL2R in preventing rejection incidence, no randomized trials have shown a benefit in long-term graft- or patient survival with rATG compared with IL2RA. Certainly the big impact on rejection rates reduces resource utilization and hence warrants the higher costs with ATG use over IL2RA.

Liver Transplantation

As an immune-privileged organ, liver transplantation is associated with the lowest utilization of induction therapies. In fact, use of induction therapies in liver transplantation remains controversial (Moini et al. 2015). Calcineurin inhibitor induced nephrotoxicity presents a big hurdle to long-term morbidity-free survival in this patient population. In fact, up to 20% of liver transplant recipients develop chronic kidney disease by year 5 post-transplant and if end-stage renal disease with dependence on dialysis is achieved, patient mortality increases 4.5-fold (Post et al. 2005). As such, induction therapies have been studied as part of CNI sparing or delayed CNI initiation strategies. Multiple small randomized controlled trials studying the use of IL2RA as renal sparing strategy with delayed CNI introduction have shown no clinically significant difference in rejection rates (although some studies achieved statistical significance) and variable impact on renal function preservation (Post et al. 2005; Moini et al. 2015). Furthermore, studies to assess impact of ATG induction have been limited by small sample size and disparate study designs, precluding any definitive conclusion to be drawn regarding its benefit in the LT population.

Additionally, antibody induction therapy has been used in liver transplant recipients as a steroid sparing strategy. IL-2RA induction therapies have been associated with less frequent diabetes mellitus, less CMV infections, and higher glomerular filtration rate vs rates observed with corticosteroids induction therapy. Similarly, ATG induction has been applied successfully with no observation of more severe recurrence of hepatitis C infection. Furthermore, unlike in kidney transplant patients, there is insufficient evidence linking ATG use with heightened risk of PTLD (Bittermann et al. 2019).

Heart and Lung Transplantation

In heart and lung transplantation, there are no randomized placebo-controlled trials comparing induction strategies with IL2RA versus ATG versus standard triple therapy.

Only ~50% of heart transplant programs currently employ a strategy of induction therapies (Colvin et al. 2021). A meta-analysis of randomized controlled trials and observational studies showed that use of any type of induction therapy did not reduce risk of moderate-to-severe rejection, all-cause death, infection, and cancer compared to no antibody induction therapy (Briasoulis et al. 2018). The use of IL2RA was associated with significantly higher risk of moderate-to-severe rejection when compared to ATG but similar risk of death, infections, and cancer. Another analysis by Ansari et al. based on data from the ISHLT registry demonstrated in a multivariable Cox model that basiliximab was associated with increased mortality over 3-year follow-up with HR 1.22 compared to ATG use (Ansari et al. 2015a, b). None of these analyses employ matching of patients for immunological risk. There is an

ongoing single-center prospective randomized study which is exploring the use of ATG induction vs placebo in a lower immunological risk cohort (defined as having PRA <25%) (NCT03292861).

There are only two multicenter, randomized studies comparing basiliximab to rATG on the background of maintenance therapy with cyclosporine, mycophenolate mofetil, and steroids. At a six-month follow-up, the basiliximab was equally protective against rejection as rATG (Carrier et al. 2007; Mattei et al. 2007). Basiliximab was better tolerated than ATG and there was a higher rate of infectious deaths in the rATG group (Carrier et al. 2007; Mattei et al. 2007). On the other hand, the rATG induction was associated with higher rates of CMV viremia (Carrier et al. 2007; Mattei et al. 2007).

Similarly, in lung transplantation, no large, prospective, randomized, placebo-controlled trials exist to compare the risks and benefits of induction therapy with IL2RA or ATG compared with conventional immunosuppression. Current evidence suggests that the induction therapy may be associated with better outcomes, although controversy exists. Induction therapy may be associated with better outcomes including reduced rates of acute rejection, bronchiolitis obliterans syndrome and possibly may improve graft survival (Scheffert and Raza 2014). In a single-center study use of basiliximab as a renal sparing strategy no difference was found in rates of acute rejection or chronic lung allograft dysfunction in those patients that received basiliximab vs those who did not, however more patients in the basiliximab group died at 1 year (Linder et al. 2021). ISHLT Registry Study of 3,970 adult lung transplant recipients suggest that IL2RA and ATG are each associated with a survival benefit following lung transplant. Those treated with IL2RA had better graft survival than those treated with ATG and those who did not receive induction (Hachem et al. 2008).

2.2 Other Induction Therapies

2.2.1 Alemtuzumab

Alemtuzumab is a humanized rat monoclonal antibody targeting the CD52 cell surface glycoprotein, which is present almost exclusively on lymphocytes as well as natural killer (NK) cells and macrophages (Vathsala et al. 2005). CD52 binding induces cell lysis and profound immune cell depletion. It has been shown that recovery of B cells occurs between 3 and 6 months and that of T cells – between 12 and 24 months after administration (Watson et al. 2005). The rationale for its use was to allow the application of steroid-free regimen. Additionally, upon lymphocyte reconstitution, there is a phenotypic shift in the T-cell population towards greater proportion of $CD4^{+}CD25^{high}$ cells and in the B cells – towards IgM-producing naïve B cells (Enderby and Keller 2015). This lymphocyte profile has been found to be similar to stable immunosuppression-free kidney recipients, hence postulating whether alemtuzumab could pave the way for immunological tolerance. To that end, alemtuzumab was tested in a randomized controlled trial and compared to basiliximab or thymoglobulin as an induction immunosuppressant as part of an

early steroid-withdrawal regimen in kidney transplant recipients (Hanaway et al. 2011). Alemtuzumab demonstrated lower rejection rates at 1 year when compared to conventional induction therapy with basiliximab or thymoglobulin in kidney transplant recipients. Alemtuzumab demonstrated superiority in rejection rates among low-risk immunologic risk patients compared to basiliximab, but similar rejection rates when compared to thymoglobulin in high-risk kidney transplant recipients. Similarly the 3C study in kidney transplant recipients examined the outcomes with alemtuzumab-based induction treatment (i.e., alemtuzumab followed by low-dose tacrolimus and mycophenolate without steroids) versus basiliximab-based induction treatment (basiliximab followed by standard-dose tacrolimus, mycophenolate, and prednisolone) (Group CSC et al. 2014). At 6 months follow-up the alemtuzumab group showed reduced risk of biopsy-proven acute rejection across broad range of patients. In a meta-analysis of four randomized controlled trials in renal transplantation, alemtuzumab induction appeared to decrease acute rejection at a cost of increased cytomegalovirus disease despite early steroid withdrawal in three of the studies. Patient-centered outcomes including reduced death or lower toxicity did not appear to be improved (Hill et al. 2017).

Unlike kidney transplant recipients, the data on alemtuzumab use in other SOT is limited. In liver transplantation alemtuzumab with minimization of maintenance immunosuppression using tacrolimus monotherapy was associated with comparable graft survival to patients receiving conventional therapy, although its use in patients with a history of hepatitis C was associated with higher rate of complication and death in one study (Marcos et al. 2004). Small single-center observational studies in HT recipients have shown lower rejection risk with its use compared to standard immunosuppression (Gale et al. 2019). In heart transplantation, induction therapy with alemtuzumab resulted in a similar 12-month survival compared to no induction, but a greater freedom from rejection despite lower calcineurin levels and without the use of steroids (Teuteberg et al. 2010). In a meta-analysis of observational studies in heart and lung transplant recipients, alemtuzumab use was associated with lower rejection rates when compared with conventional induction therapy agents (antithymocyte globulin, basiliximab, and tacrolimus) (Li et al. 2018).

In a randomized trial 60 patients undergoing lung transplantation were followed up for 2 years and assessed for renal function as the prespecified primary end-point. Secondary end points included survival, refractory or recurrent acute rejection, and freedom from bronchiolitis obliterans syndrome (BOS). The study demonstrated no difference in renal function between the two induction groups at 6 and 12 months post-transplant. Additionally, there were no differences noted in any of the secondary end points. Patients receiving alemtuzumab induction however experienced significantly less grade $\geq$ A2 acute rejection within the first post-transplant year when compared to those receiving ATG (Jaksch et al. 2014). In a retrospective single-center analysis including 446 lung transplant recipients, of which 52% received alemtuzumab, 11% received ATG, and 37% received no induction therapy, the alemtuzumab group had the lowest rate of chronic kidney insufficiency and infection in the first year (Benazzo et al. 2019). Improved survival and low rates of ACR, lymphocytic bronchiolitis, and chronic lung allograft dysfunction (CLAD)

were found in the group receiving any induction therapy. Retrospective UNOS Registry Study in 6117 lung transplant recipients demonstrates longer median survival for alemtuzumab and basiliximab versus no induction. Recipients of alemtuzumab had a lower incidence of BOS at 5 years (Furuya et al. 2016). There is however concern that these potent lymphocyte-depleting agents may reduce regulatory cell populations important in tolerance and lower maintenance immunosuppressive regimens commonly used with alemtuzumab may result in increased rates of late rejection (Todd and Palmer 2014).

Common major adverse effects include leukopenia which can last several months, anemia, thrombocytopenia, and infusion-related reactions.

2.2.2 Costimulatory Blockade (Belatacept)

The costimulation pathway is critical for T-cell activation. CD28 is a crucial costimulatory molecule required for T-cell activation, and CTLA-4, its homologue, attenuates T-cell activation. Both CD28 and CTLA-4 bind to CD80 and CD86 that are found on antigen-presenting cells. Inhibition of CD80/86 receptors with a high-affinity molecule blocks costimulation and inhibits T-cell activation (Enderby and Keller 2015). Belatacept is a fusion protein composed of the human IgG constant domain linked to the modified extracellular moiety of cytotoxic T-lymphocyte–associated antigen 4, a homolog of CD28. Belatacept binds to CD80 and CD86 on antigen-presenting cell and inhibits CD28-mediated T-cell costimulation which is essential for T-cell activation and graft rejection. Belatacept was approved by the US FDA in 2011 for use in combination with basiliximab, mycophenolate mofetil, and corticosteroids for rejection prophylaxis in adult renal transplant recipients who are EBV seropositive (Enderby and Keller 2015; Masson et al. 2014). The premise behind introducing belatacept early post kidney transplantation was to delay or spare CNI use thus minimizing toxicities commonly associated with these agents, including nephrotoxicity, hyperglycemia, hypertension, among others (Masson et al. 2014). In the Belatacept Evaluation of Nephroprotection and Efficacy as First-line Immunosuppression Trial (BENEFIT), a 3-year, randomized, active-controlled, parallel group, multicenter phase 3 trial and the BENEFIT-EXT trial of recipients of extended criteria donors, use of de novo belatacept was associated with improved renal preservation, comparable graft and patient survival, with preservation of renal function maintained long-term (Vincenti et al. 2010a, b; Durrbach et al. 2010). In the kidney transplant population, belatacept has been shown to reduce incidence of de novo DSA formation and to improve long-term renal function and graft survival (Masson et al. 2014; Vincenti et al. 2016; Bray et al. 2018a, b). However, the rates and severity of acute cellular rejection are higher compared with cyclosporine-based regimen and they usually occur in the first 3 months post transplant (Bray et al. 2018b). Importantly, however, the increased rate of early rejection has not been shown to negatively impact 7-year patient or graft survival (Vincenti 2016). Additionally, based on 7-year outcomes data belatacept has been shown to have comparable safety profile to traditional immunosuppressive regimen (Vincenti 2016).

Subsequently, the multicenter, randomized Belatacept Early Steroid Withdrawal Trial (BEST) was conducted exploring the feasibility for simultaneous CNI

avoidance and early corticosteroid withdrawal in kidney transplant recipients (Kaufman et al. 2021). The study tested 3 comparator groups – alemtuzumab/belatacept, rATG/belatacept, or rATG/tacrolimus. At 2 years follow-up, there were no significant differences in the primary composite end-point of rates of death, graft loss, or eGFR <45 mL/min/1.73 m^2 among groups. However, a significantly greater proportion of tacrolimus-treated patients had an eGFR <45 mL/min/1.73 m^2. The proportion of corticosteroid-free patients at month 24 was similar across the groups (Kumar et al. 2017).

Drawing on the success of belatacept in kidney transplantation, a phase II study was conducted in de novo adult liver transplant recipients comparing 2 treatment doses of belatacept in combination with mycophenolate mofetil to a tacrolimus-based regimen (Klintmalm et al. 2014). Patients were more than 2 years post-LT at the time. At 12 months, belatacept treated patients had higher rates of acute rejection as compared with the control group and in two of three belatacept groups there were higher rates of death due to infection and graft loss relative to the standard-of-care tacrolimus and MMF control group. The glomerular filtration rate (GFR) was better in the belatacept group as compared with the tacrolimus group. The trial was terminated early due to the rate of adverse outcomes seen in the belatacept group (Klintmalm et al. 2014). Belatacept is therefore not recommended in liver transplantation. Concerns for PTLD also have led to a black box warning against its use in Epstein-Barr seronegative recipients.

There is less data for efficacy of belatacept as part of induction therapy in other solid organ transplantation. Currently, there is an ongoing pilot clinical study to determine the safety of belatacept in de novo heart transplant recipients (NCT04477629). It seeks to enroll 10 primary heart transplant recipients, EBV seropositive, who will receive belatacept in addition to mycophenolate mofetil, corticosteroids, along with tacrolimus tapering regimen over the 9 months post OHT. Another study seeks to assess efficacy of belatacept in improving renal function in heart transplant recipients with renal dysfunction at 3 months post transplant in conjunction with CNI withdrawal (NCT04180085).

3 Strategies to Neutralize the Effect of Pre-Formed Antibodies

The deleterious role of pre-formed donor specific antibodies (DSAs) has been well established. Strategies to reduce their impact on post-transplant outcomes have been piloted in the kidney transplant recipients. Various combinations of therapies have been applied in this setting. Therapeutic plasma exchange (TPE) entails the physical removal of circulating alloantibodies but its effect is short-lived with rebound uprise of antibody production after cessation (Valenzuela and Reed 2017). Hence, it is combined with other more durable therapies. Intravenous immunoglobulin (IVIG) is a pooled preparation of immunoglobulins with pleotropic targets (Valenzuela and Reed 2017). IVIG inhibits activation of innate immune cells, neutralizes complement, modulates B- and plasma cells, enhances Treg function, induces apoptosis of

activated effector T cells, and downregulates production of inflammatory cytokines (Valenzuela and Reed 2017). Rituximab is a chimeric anti-CD20 monoclonal antibody which directly inhibits B-cell proliferation, induces apoptosis, and reduces the production of antibodies (Valenzuela and Reed 2017).

Loupy et al. compared two induction strategies in kidney transplant recipients with pre-formed DSAs: group 1 received standard triple therapy with addition of four high-dose IVIG infusions and group 2 received the same dose of IVIG with additional rituximab and PP (Loupy et al. 2010). At 1 year post-transplant, group 2 was characterized by lower rate of transplant glomerulopathy, lower rate of chronic AMR, and better glomerular filtration rate (Loupy et al. 2010). A retrospective study of highly sensitized kidney transplant recipients (crossmatch positive or DSA positive) treated with IVIG and rituximab induction therapy demonstrated higher rates of AMR in sensitized recipients compared to low-risk kidney transplant controls, but similar patient or graft survival at 6-year follow-up (Kahwaji et al. 2016). In another study, the combination of rituximab with rATG induction therapy in highly sensitized patients (mean class I panel reactive antibodies (PRA) $> 80\%$) exhibited better graft survival at 5 years compared to rATG induction therapy alone (Laftavi et al. 2015). A study of HLA-incompatible recipients (mean cPRA $= 80\%$, repeat HLA mismatches (80%), CDC positive, FCXM positive, or DSA positive) found that rituximab induction had no effect on AMR rates or 5-year alllograft survival compared to patients transplanted without rituximab (Jackson et al. 2015). Despite the observed acceptable outcomes with rituximab use, it is not widely used due to safety concerns, such as susceptibility to bacterial infections, post-transplant lymphoproliferative disease (PTLD), hypogammaglobulinemia, and progressive multifocal leukoencephalopathy (PML) (Jackson et al. 2015).

In the field of cardiac transplantation, data on IVIG use are more limited and originate from observational data alone. When used in combination with TPE, IVIG has been reported to reduce the incidence of rejection after transplantation across a positive crossmatch (Leech et al. 2006; Pisani et al. 1999). There is paucity of evidence for use of the above antibody-directed strategies in other solid organ transplantation. Moreover, in liver transplantation donor HLA typing and hence, pre-formed DSA identification is not routinely performed.

4 Monoclonal and Polyclonal Antibodies Outside Induction Therapy

In transplantation, activation of both innate and adaptive immune systems leads to injury and rejection of the allograft, although the latter plays a predominant role through both initial injury and establishment of a memory response which allows a rapid, targeted, and robust response to antigenic re-exposure. Both cellular and humoral mechanisms form critical components of allograft rejection and are important therapeutic targets. Alloantigens (HLA Class I and II molecules) derived from the graft are presented to naïve T cells which mature to Th helper cells in the germinal centers of regional lymph nodes and spleen of the recipient.

Dendritic cells, which act as sentinels in the peripheral tissues, recognize and pick up alloantigens, process them, and present them to T cells. Antigen presentation occurs in conjunction with HLA class I molecules to CD 8+ cytotoxic T cells and with HLA class II molecules to CD4+ Th cells and occurs in combination with costimulatory molecules (B7 and CD40). Donor antigen-presenting cells may also directly present antigens to T cells. T cells of appropriate specificity respond to the antigen, which causes either direct cytotoxicity (CD8+ T cells) (acute cellular rejection (ACR)) or secretion of cytokines (Th cells) that will stimulate B lymphocyte response or recruit other inflammatory cells. B cells provide humoral immunity by secreting antibodies specific for the pathogen or antigen. Th cell activation occurs through production of a number of cytokines including IL6 and IL21. Alloantibody then migrates to the graft and initiates injury through complement activation, antibody dependent cellular cytotoxicity (ADCC) or direct interaction of antibody with cell surface antigens with resultant antibody-mediated rejection (AMR).

The development of polyclonal and monoclonal antibodies has provided an armamentarium for both prevention and treatment of allograft rejection (Fig. 1). These therapies have targeted specific critical points in the immune cascade. Many agents have been developed for treatment of autoimmune or other diseases and have been adapted in the transplant arena with variable degrees of success. Therapeutic windows are variable with many antibody preparations associated with toxicity and importantly efficacy tends to be highly variable and, in many cases, limited. Clinical use of these agents is further hampered by lack of clinical trials.

4.1 Prophylactic Therapy

Antibody preparations have been widely used for prevention of hyperacute or acute rejection at transplant, historically with the hope of inducing graft tolerance, hence the misnomer “induction therapy” (see above). Over the years, several preparations have had a varying degree of popularity, with some now only of historical interest. The majority of these have been potent cytolytic therapies focused on abrogating the cellular response to the implanted allograft. However, as use of induction therapy is not universal especially for certain organs, there has been limited randomized clinical data to suggest incremental efficacy with this approach.

4.2 Intravenous Immunoglobulin (IVIg)

IVIg are commercial preparations of immune globulin that consists of intact IgG molecules pooled from the plasma of thousands of healthy blood donors. IVIg has multiple and complex immune effects. These include neutralization of circulating antibodies, inhibition of B-cell activation and maturation through upregulation of inhibitory B-cell FcγRIIB, inhibition of B-cell growth factors, and cross-linking B-cell receptor and FcγRIIB, which reduce antigen presentation activity and induce B-cell apoptosis (Chih and Patel 2016). Most of IVIg products are approved for the

treatment of primary humoral immunodeficiency and immune thrombocytopenic purpura.

In a randomized clinical trial in sensitized patients awaiting renal transplantation, repeated infusions of IVIg significantly reduced PRA and improved transplant rates compared to placebo (Jordan et al. 2004). In another randomized trial a significant survival benefit was demonstrated in HLA-sensitized patients who underwent desensitization (pretransplant and perioperative plasma exchange with IVIg) prior to living kidney transplant when compared to sensitized patients who waited for a compatible organ without desensitization (Montgomery et al. 2011). Multiple observational studies reported variable efficacy of IVIg ± plasmapheresis in reducing pretransplant sensitization (Shehata et al. 2010).

Early studies demonstrated that IVIg and plasmapheresis decreased PRA and shortened the waiting time on the list for sensitized heart transplant candidates (Leech et al. 2006; Pisani et al. 1999; John et al. 1999, 2001). True efficacy of IVIg is difficult to quantify due to the variability in sensitization of treated patients, inherent variability in IVIg polyclonal preparations and frequent use of concurrent therapies (see below).

IVIg is usually well tolerated although various adverse effects have been reported. Infusion reactions may occur, minimized with corticosteroid, antihistamine, and analgesic prophylaxis. Renal impairment has been described with sucrose containing preparations, as have thrombosis and transfusion-related acute lung injury. Hemolytic anemia is caused by the presence of blood group antibodies, namely, anti-A, anti-B in IVIG products, and is an under-recognized complication of IVIg therapy.

4.3 Muromonab-OKT3

Muromonab is mostly of historical interest as it is no longer clinically available. Clinical data are limited with no survival benefit compared to other treatments, although its use facilitated delayed calcineurin inhibitor therapy in renal and hepatic transplant recipients with lower rejection rates. With respect to use in steroid resistant rejection in renal transplantation, muromonab appeared equivalent or less effective at preventing graft loss compared to ATG (see below) but the latter was better tolerated (Mariat et al. 1998; Kainz et al. 2010).

4.4 Antithymocyte Globulins

While ATG has been used for steroid resistant and recurrent rejection following renal transplantation, no prospective data on using ATG in acute antibody-mediated rejection is available. In a small retrospective study of seven patients developing early acute AMR, use of pulse steroids, ATG and plasma exchange resulted in improvement in graft function (Shah et al. 2004). Given the absence of effectiveness of ATG on plasma cells and AMR rates as high as 40% in desensitized patients

despite ATG induction, the use of ATG in the treatment of AMR is limited to being part of combination therapy. The use of ATG has been associated with increased risk of opportunistic infections and malignancy (Malvezzi et al. 2015).

In a retrospective single-center analysis of treatment of corticosteroid resistant acute cellular rejection after liver transplantation, use of ATG in 20 recipients was associated with resolution of biopsy confirmed rejection in 90% of cases with excellent short-term outcomes. However, some liver transplant recipients failed to respond, and 3-year survival was reduced, even in those who responded to antithymocyte globulin (Palmer et al. 2018).

4.5 Alemtuzumab

With regard to the use of alemtuzumab for acute rejection, the most extensive reported experience is in renal transplantation (van der Zwan et al. 2020). Outcomes of patients treated with alemtuzumab for acute kidney allograft rejection were compared with that of patients treated with rabbit ATG for acute rejection. Outcomes of 116 alemtuzumab-treated patients were compared with those of 108 propensity score matched patients treated with rATG for acute rejection. Patient and allograft survival following treatment with either regimen were not different. Infection-free survival after alemtuzumab treatment was superior compared with that of rATG-treated patients and infusion-related adverse events occurred less frequently after alemtuzumab treatment.

Alemtuzumab has been used in treatment of acute rejection in lung transplantation refractory to corticosteroids and ATG and for treatment of bronchiolitis obliterans syndrome (BOS) (Reams et al. 2007). In 12 patients with refractory acute rejection, use of alemtuzumab resulted in significant improvement in histological rejection scores. At 2 years, freedom from BOS was observed in 65% of patients. In ten patients treated with alemtuzumab for BOS, although there was no statistically significant change in forced expiratory volume in 1 s (FEV1) before and after alemtuzumab treatment, stabilization or improvement in BOS grade occurred in 70% of patients. Patient survival 2 years after alemtuzumab for BOS was 69%. With an aggressive infection prophylaxis protocol, despite a dramatic decline in CD4 counts in alemtuzumab-treated patients, only one patient developed a lethal infection.

4.6 Interleukin-2 Receptor Antagonists

Most experience with these agents is with their use as induction agents for the prevention of rejection where CD25 blockade may be more effective prior to production of IL2 following T-cell activation in the rejection cascade. In renal transplantation, while it appears effective in preventing rejection in low-risk patients, in an early randomized study of patients at high risk for rejection or delayed graft function, ATG was found to be superior to basiliximab in reducing the incidence and

severity of acute rejection but not the incidence of delayed graft function (Brennan et al. 2006). In a retrospective analysis, an increased incidence of acute rejection was observed in low-risk renal transplant recipients with PRA >10% (Pereira et al. 2016). While evidence of use of basiliximab in heart transplantation is sparse, in one systematic review, there appeared to be no benefit of its use with respect to survival or reduction in acute rejection, with some studies showing superiority of ATG over basiliximab in the prevention of acute rejection (Moller et al. 2008).

4.7 Rituximab

Rituximab is a genetically engineered chimeric murine/human monoclonal IgG1 kappa antibody directed against the CD20 antigen, which is a surface antigen present on B cells. Therefore, it acts by depleting normal as well as pathogenic B cells while sparing plasma cells and hematopoietic stem cells as they do not express the CD20 surface antigen. In the USA, it is approved for the treatment of non-Hodgkin's lymphoma, chronic lymphocytic leukemia, and rheumatoid arthritis. Rituximab mediates B-cell lysis through ADCC and CDC. Treatment is generally well tolerated although infusion-related reactions may occur, manageable with acetaminophen, antihistamine, and corticosteroid premedication or by slowing the infusion rate.

Rituximab in solid organ transplantation has been mainly used as part of a desensitization strategy, for attempting empiric management of AMR and treatment of post-transplant CD20 positive lymphoproliferative disease. Patients sensitized to HLA antigens have a longer time to transplantation, increased wait-list mortality and greater risk of rejection after transplantation. Rituximab has been used in various settings to decrease circulating HLA antibodies in highly sensitized patients to facilitate transplantation. In renal transplantation, the use of rituximab in combination with intravenous immunoglobulin (IVIG) in 20 highly sensitized patients awaiting transplantation resulted in a decrease in mean PRA (77 $\pm$ 19% to 44 $\pm$ 30%), resulting in a significantly decreased mean time to transplant (from 144 $\pm$ 89 months to 5 $\pm$ 6 months in 16 of 20 patients transplanted) with excellent 1 year patient and graft survival (94% and 100%, respectively) (Vo et al. 2008). In a larger study of 207 patients from the same group, use of rituximab and IVIG for desensitization in patients with DSA or PRA $\geq$80% led to a transplant rate of 71%, 95%, and 87.5% patient and graft survival, respectively, 22% with AMR and 5.5% with allograft loss due to AMR (Vo et al. 2013). The use of rituximab and IVIG in conjunction with the 26S proteasome inhibitor bortezomib which targets plasma cells in 19 patients awaiting renal transplantation was shown to increase transplant rate to 42% compared to 23% for untreated controls, increasing the probability of deceased donor kidney transplantation (hazard ratio [HR], 46.9; 95% confidence interval [CI], 4.5–634.2; $P = 0.004$) (Jeong et al. 2016).

Rituximab has also been used at the time of transplantation for highly sensitized patients in the setting of positive cytotoxic or flow cytometric crossmatch, positive DSA but negative crossmatch and in patients with high PRA. Efficacy of therapy is difficult to discern as various concomitant therapies including plasmapheresis and

induction regimens were used. In one meta-analysis of 589 highly sensitized patients awaiting renal transplantation, rituximab induction (n = 312) pretransplant lead to significantly fewer AMR episodes and higher 1-year graft survival rates (Zhao et al. 2014). However, in a randomized, double blind, placebo-controlled study of 280 patients randomized to a single-dose rituximab or placebo during transplant surgery with standard triple immunosuppression, the biopsy-proven acute rejection rate at 6 months was similar in the two groups (van den Hoogen et al. 2015).

In heart transplantation, data on the use of rituximab is limited. In a retrospective single-center analysis of 21 patients with PRA > 10%, plasmapheresis was variably utilized with IVIG and with or without rituximab. These treated patients were compared with untreated patients with PRA > 10% ($N = 74$) or patients with PRA < 10% ($N = 428$). Treatment resulted in a decrease in mean PRA from 71% to 31% with all patients able to proceed to transplantation. Desensitization therapy was associated with higher rates of AMR but not ACR at 1-year but comparable 5-year survival, freedom from cardiac allograft vasculopathy and non-fata major adverse cardiac events and treated infection compared to the other two groups (Kobashigawa et al. 2011). However, the CTOT-11 randomized trial raised concerns about the use of rituximab in heart transplantation. Rituximab as an induction therapy in non-sensitized patients was associated with a marked and unexpected increase in cardiac allograft vasculopathy progression as assessed by intravascular ultrasound compared to placebo (Starling et al. 2019). Potential mechanisms for this observed effect may relate to elimination of regulatory B cells.

4.8 Obinutuzumab

Obinutuzumab is a fully humanized genetically engineered CD-20 directed monoclonal antibody targeting B-cell lysis predominantly through ADCC, directly activating death signaling pathways and but also through activation of the complement cascade. Obinutuzumab induces increased B-cell depletion relative to rituximab and may therefore be more effective for desensitization. In an initial phase 1 study of the safety, pharmacokinetics, and pharmacodynamics of obinutuzumab in 20 highly sensitized patients with end-stage renal disease, obinutuzumab plus IVIG resulted in profound peripheral B-cell depletion and also appeared to reduce B-cell populations in retroperitoneal lymph nodes. However, reductions in anti-HLA antibodies, number of unacceptable antigens, and cPRA score were limited and not clinically meaningful for most patients (Redfield et al. 2019).

4.9 Tocilizumab

Tocilizumab is a humanized monoclonal antibody that targets both soluble and membrane-bound forms of the Interleukin-6 receptor (IL-6R). IL-6 is a major cytokine which functions as a master regulator of inflammation. It plays a

fundamental role in the development, maturation, and activation of not only T cells but also B cells and plasma cells (Tanaka and Kishimoto 2014; Jordan et al. 2017). It promotes Th2 cell differentiation and the development of a humoral immune response. Diseases have been associated with excessive IL-6 production and are generally characterized by unregulated antibody production and autoimmunity (Hunter and Jones 2015). Clinically, tocilizumab is approved for the treatment of rheumatoid arthritis, giant cell arteritis, polyarticular and systemic juvenile idiopathic arthritis, cytokine release syndrome and has recently been recommended for use in certain hospitalized patients with rapid respiratory decompensation due to COVID-19.

IL-6 is a major cytokine upregulated during allograft rejection and a mediator of allograft injury (Jordan et al. 2020). In conjunction with costimulation signal blockade, its inhibition increased allograft tolerance by limiting the differentiation of effector cells and by promoting the migration of Tregs into the grafts (Zhao et al. 2012). IL-6R blockade resulted in decreased antibody production by both splenic and bone marrow plasma cells including anti-HLA antibodies (Jordan et al. 2017).

Initial experience in renal transplantation appears promising. Ten broadly sensitized patients awaiting kidney transplant refractory to IVIg and rituximab were treated with IVIg and tocilizumab in an open label single-arm phase I/II clinical trial. DSA strength was significantly reduced not only at transplant but also at 12 months after transplantation (Vo et al. 2015). In a study of 36 kidney transplant recipients with chronic active AMR and transplant glomerulopathy refractory to conventional therapy treated with tocilizumab, an improvement in the histological features of chronic AMR and a modest decline in the MFI of immunodominant DSA beginning at 24 months after the initiation of the therapy were observed (Choi et al. 2017).

Experience with tocilizumab in thoracic organ transplantation is evolving. A randomized multicenter clinical trial is ongoing to evaluate the use of tocilizumab induction versus placebo with standard triple immunosuppressive regimen in low immunological risk heart transplant recipients (NCT03644667).

In autoimmune disorders, use of tocilizumab has been associated with an increased risk of serious infections, including tuberculosis, bacterial, invasive fungal, viral, and other opportunistic infections. Hepatic dysfunction including late serious hepatic injury and hematologic toxicities with neutropenia and thrombocytopenia have also been described.

4.10 Clazakizumab

Clazakizumab is an investigational aglycosylated humanized rabbit monoclonal antibody against the IL-6 molecule. A recent phase 2 randomized clinical trial of the drug in late AMR following renal transplantation in 20 patients suggests a potentially beneficial effect of clazakizumab on AMR activity and progression but the trial was withdrawn due to a 25% incidence of serious infectious events (Doberer et al. 2021).

4.11 CD38 Antibodies

As alloantibody-producing plasma cells express CD38 at a higher level than other CD38+ hematopoietic cells, antibodies targeting the CD38 molecule appear an attractive choice for management of solid organ transplant recipients with allosensitization.

CD38 antibodies induce a profound depletion of CD38+ plasma cells and represent important therapies for the treatment of multiple myeloma. Daratumumab is a fully human IgG1-kappa monoclonal antibody which has multiple effects including Fc-dependent immune-effector mechanisms (ADCC and CDC) and direct effects including induction of apoptosis, as well as inhibition of CD38 ectoenzyme function, which may lead to disruption of the PCs niche. These effects are associated with profound and sustained CD38+ cells depletion, mostly plasma cells and NK cells. Isatuximab is a chimeric IgG1-kappa anti-CD38 antibody which has stronger direct effects than daratumumab but lower ability to induce Fc-dependent immune-effector mechanisms. Currently, only few case reports have been published regarding the use of CD38 antibodies for desensitization in patients awaiting transplantation or for treatment of ABMR (Joher et al. 2021).

4.12 Eculizumab

Eculizumab is a recombinant humanized monoclonal antibody which blocks activation of terminal complement components. It binds specifically to the terminal complement protein C5, inhibiting its cleavage into C5a and C5b, thereby preventing the release of the inflammatory mediator C5a and the formation of the membrane attack complex (C5b-9).

As stated above, complement-dependent pathways play a key role in the AMR-induced acute allograft injuries. Theoretically, inhibition of the terminal complement pathway may have several advantages over the proximal inhibition, chiefly, preservation of the main immune-protective functions of the complement cascade.

Eculizumab is approved for the treatment of paroxysmal nocturnal hemoglobinuria, atypical hemolytic uremic syndrome, generalized myasthenia gravis with anti-acetylcholine receptor antibody positive, and neuromyelitis optica spectrum disorder with anti-aquaporin-4 antibody positive.

Eculizumab has been evaluated in the prevention of AMR and graft loss in B-cell positive flow crossmatch kidney transplant recipients from living donors (Stegall et al. 2011). Biopsy-proven AMR in the first 3 months post-transplant in 26 highly sensitized recipients receiving eculizumab post-transplant was compared to a historical control group of 51 sensitized patients treated with a similar plasma exchange-based protocol without eculizumab. The incidence of AMR was 7.7% in the eculizumab group compared to 41.2% in the control group ($p = 0.0031$). On 1-year protocol biopsy, transplant glomerulopathy was found to be present in 6.7% eculizumab-treated recipients and in 35.7% of control patients ($p = 0.044$).

However, beyond 1 year eculizumab did not appear to prevent transplant glomerulopathy in patients with persistently high B-cell flow crossmatch (Cornell et al. 2015). In a single-arm open label trial, 80 patients transplanted with pre-formed DSA were treated with a 9-week eculizumab course with acceptable outcomes. Observed treatment failure rate (8.8%) was significantly lower than expected for standard care (40%; $P < 0.001$). At 36 months, graft and patient survival rates were 83.4% and 91.5%, respectively) (Glotz et al. 2019).

Our group recently reported a first non-randomized, open label, single-arm prospective trial of eculizumab in high immunological risk heart transplantation. The main inclusion criteria were pretransplant PRA $\geq$ 70% and high level of DSA at transplant. Terminal complement inhibition was well tolerated and associated with favorable outcomes. No patient experienced hemodynamic compromise or graft dysfunction during the first-year post-transplant. When comparing eculizumab-treated patients with plasmapheresis-IVIg treated patients at equivalent immunological risk, eculizumab was associated with a dramatically decrease in the risk of biopsy-proven AMR (Patel et al. 2021). The use of eculizumab after lung transplantation is limited to case reports of rescue therapy for severe AMR.

The use of eculizumab is associated with an increased risk of Neisseria meningitidis infections. A vaccination against N. meningitidis at least 15 days before starting therapy is required. Where this is not possible, prophylactic antibiotics should be administered until 2 weeks after completion of therapy. There have been anecdotal reports of the use of eculizumab for the treatment of AMR following renal, lung, and heart transplantation (Kittleson et al. 2021; Tan et al. 2019; Yelken et al. 2015).

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Immunosuppression and Heart Transplantation

Nilay Sutaria, Lynne Sylvia, and David DeNofrio

Contents

Abstract

Since the first human heart transplant in 1967, immense advancements have been made in the field of immunosuppression. This chapter provides an in-depth analysis of the use of immunosuppressive agents in heart transplant recipients. Evidence regarding maintenance immunosuppressive regimens, the efficacy of induction immunosuppression and corticosteroid weaning, as well as the use of distinct immunosuppression regimens within select patient populations is summarized. This chapter helps elucidate the data regarding contemporary protocols in cardiac transplantation.

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1 Introduction

It has been over 50 years since Dr. Christiaan Barnard performed the first heart transplant on December 3, 1967 in Cape Town, South Africa (Brink and Hassoulas 2009). Although the recipient was treated with immunosuppressive medications available at the time to avoid allograft rejection, the patient ultimately died a few weeks later from pneumonia. Following this landmark case, additional early attempts at heart transplantation were hindered by both limited surgical techniques, and more importantly, the lack of ideal immunosuppressive medications. With the introduction of cyclosporine in the 1980s, cardiac transplantation success rates increased dramatically making transplant a more viable treatment option for patients with end-stage heart failure, and as a result, the number of transplants performed worldwide grew dramatically. Since that time, new immunosuppressive drugs and strategies have continued to improve short and long-term outcomes for patients undergoing heart transplantation. In 2018, there were 3,408 heart transplant procedures performed in the USA and in the current era, patients have an expected median survival rate of over 13 years with an ever increasing number of patients surviving beyond 20 years (UNOS Transplant Trends 2019; Lund et al. 2017). This chapter will review the key evidence behind contemporary immunosuppression management of cardiac transplant recipients and discuss specific immunosuppressive strategies in special post-transplant populations.

2 Maintenance Immunosuppression

Immunosuppression has evolved dramatically over the last 50 years since the initial cardiac transplant procedures were attempted. In the 1970s and early 1980s, immunosuppressive regimens consisted of induction therapy with antithymocyte globulin (ATG) and intravenous methylprednisolone followed by maintenance therapy with oral prednisone and azathioprine. Survival rates were approximately 49% at 1 year and 23% at 5 years (Griepp et al. 1976). The availability of cyclosporine in the 1980s further revolutionized immune suppression and improved survival rates at 1 year by 20–25% (Myers et al. 1988). By the 1990s, maintenance regimens were primarily composed of oral cyclosporine, azathioprine, and corticosteroids. The goal of this "triple drug" immunosuppressive regimen was to minimize dose and toxicity of each drug while at the same time inhibiting multiple pathways involved in T-cell activation. Further advances were made in the

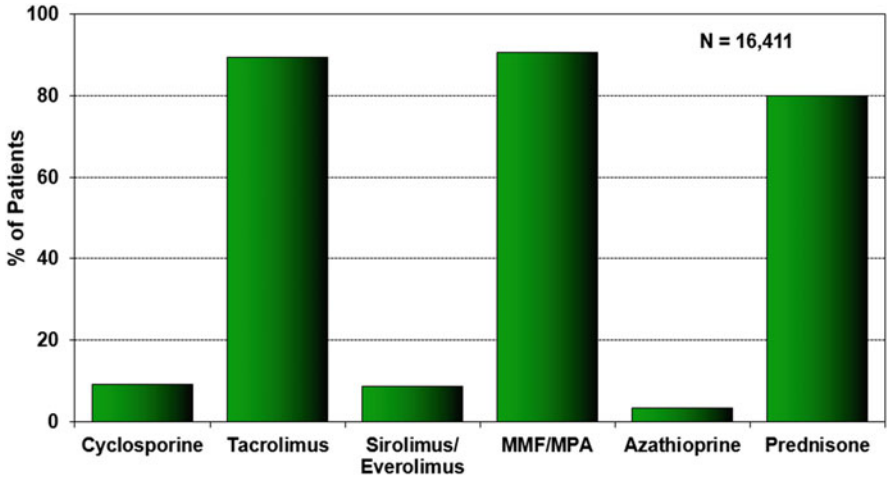

Fig. 1 ISHLT Registry of Adult Heart Transplants. Maintenance immunosuppression at time of 1 year (Follow-ups: January 2009 to June 2017). https://ishltregistries.org/registries/slides.asp

mid-1990s when both mycophenolate mofetil (MMF) and tacrolimus were approved for use in cardiac transplantation. These agents have now supplanted both cyclosporine and azathioprine in primary maintenance regimens due to improved survival rates and reduced rates of adverse side effects including cytopenias. As a result of this improved regimen, 1-year survival rates in the USA have increased to approximately 90% (Baran et al. 2007). More recently, introduction of the proliferation signal inhibitors, sirolimus and everolimus, has changed the landscape of immunosuppression and provided additional combination regimens that can be utilized in special patient populations to inhibit the development/progression of cardiac allograft vasculopathy (CAV), renal toxicity, and cancers.

Contemporary immunosuppression regimens in cardiac transplant now consist of tacrolimus, MMF, and corticosteroids (Fig. 1). Many programs attempt to wean corticosteroids within the first year as long as there is no significant rejection or other indications to limit its withdrawal. The rationale for corticosteroid weaning is to minimize the long-term side effects of chronic steroid administration which include the development of diabetes, Cushingoid body habitus, osteoporosis, and cataracts. The most recent data from the ISHLT Registry suggest that despite these attempts, approximately 80% of patients are still on some dose of corticosteroid at 1 year following transplant. This triple-drug regimen has shown to be extremely effective in multiple clinical trials demonstrating less rejection and graft failure at 1 year. Nonetheless, immunosuppression in transplant is a double-edged sword and there needs to be a balance between the level of immunosuppression and toxicity from these agents. Therefore, immunosuppression should always be individualized to take into account the patient's risk profile and comorbidities. For example, a patient with gastrointestinal side effects from MMF may benefit from a change to azathioprine, although less immunosuppressive, in order to avoid significant toxicity. Another

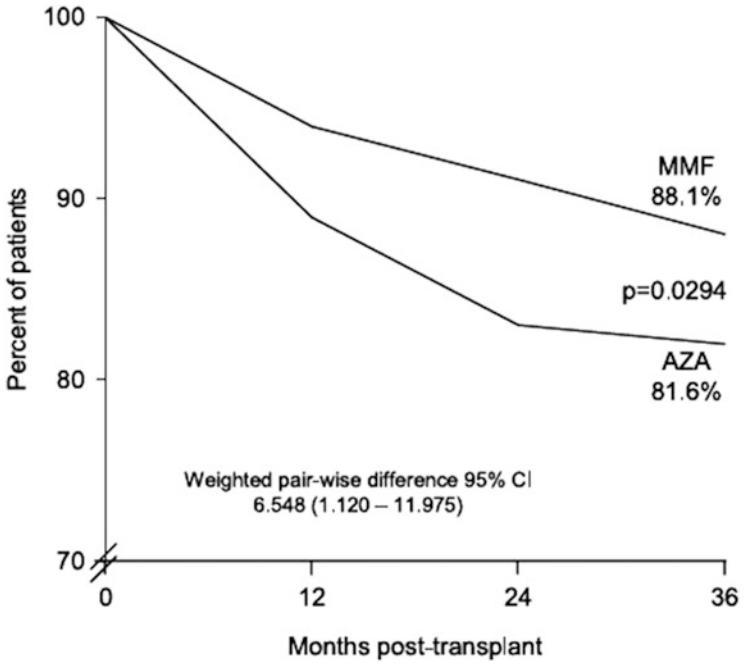

Fig. 2 Proportions of patients receiving mycophenolate mofetil (MMF) or azathioprine (AZA) surviving and not requiring retransplantation at 12. Twenty-four and thirty-six months post-transplant (treated patient population) (Eisen et al. 2005)

example would be a patient with significant renal dysfunction who may benefit from a calcineurin-free immunosuppressive regimen in order to avoid progression of their renal disease.

The introduction of MMF as a new immunosuppressive antimetabolite marked an important event in the 1990s. The heart transplant community conducted a large multicenter randomized trial comparing MMF to azathioprine (Kobashigawa et al. 1998). This double-blind, active-control trial enrolled 650 patients undergoing their first heart transplant procedure. Patients were randomized after transplantation to receive either MMF (3,000 mg/day) or azathioprine (1.5–3 mg/kg/day) in addition to cyclosporine and corticosteroids. While the intent-to-treat analysis was similar between both groups, treated-patient demonstrated a 45% reduction in 1-year mortality (6.2% versus 11.4%, $p = 0.31$) and a reduction in the number of patients with any treated rejection (65.7% versus 73.7%; $p = 0.026$) in favor of MMF. Of note, opportunistic infections, mostly herpes simplex infections, were more common in the MMF group (53% versus 43.6%; $p = 0.025$). The 36-month results of this trial were later published and continued to demonstrate a reduction in mortality in the MMF treated patients (Fig. 2) (Eisen et al. 2005). Interestingly, no significant differences between MMF and azathioprine treated patients were observed in mean maximal intimal thickness or quantitative coronary angiographic

measurements of transplant coronary vasculopathy. This trial served as pivotal data supporting the immunosuppressive superiority of MMF over azathioprine in heart transplantation.

Another important milestone in the field of immunosuppression was the introduction of tacrolimus, which quickly supplanted the use of cyclosporine once studies highlighting its benefits were conducted. Initially, two small multicenter trials were conducted comparing tacrolimus to cyclosporine. In the European Heart Transplantation Pilot Study, 82 patients were randomized to treatment with either tacrolimus or cyclosporine-based therapy. Although this study only had a small number of patients enrolled, no significant differences were found in survival or freedom of rejection rates. In this study, tacrolimus appeared to possess an advantage with regard to a reduced requirement for antihypertensive therapy (59% versus 87.5%, $p = 0.025$) (Reichart et al. 1998). Taylor et al. published a small US experience of 85 patients randomized to receive cyclosporine- or tacrolimus-based regimens. Similar to the European trial, there was no difference in rejection at 1 year between the two groups (Taylor et al. 1999). Furthermore, tacrolimus therapy was associated with less hypertension and hyperlipidemia. No difference was noted in renal function between the two groups. Following these initial trials, several other studies were conducted examining the safety and effectiveness of a tacrolimus-based compared to cyclosporine-based regimens. A summary of these studies and findings is highlighted in Table 1.

Grimm et al. and Kobashigawa et al. published the largest of these studies in 2006 (Grimm et al. 2006; Kobashigawa et al. 2006a). Grimm et al. randomized 314 heart transplant recipients following antibody induction therapy to receive either tacrolimus or cyclosporine in combination with azathioprine and corticosteroids. The primary end point, incidence of first biopsy-proven acute rejection at 6 months (ISHLT >1B), was 54% for tacrolimus treated patients and 66.4% for cyclosporine treated patients ($p = 0.029$). Survival rates were 92.9% for tacrolimus and 89.8% for cyclosporine at 18 months. Differences were noted between tacrolimus and cyclosporine treated patients with regard to relevant adverse events such as new-onset diabetes (20.3% versus 10.5%), hypertension (65.6% versus 77.7%), and dyslipidemia (28.7% versus 40.1%). In addition, there were no differences appreciated in infection rates and renal function at 18 months (Grimm et al. 2006). Kobashigawa et al. randomized 343 heart transplant recipients to receive corticosteroids and either tacrolimus/sirolimus, tacrolimus/MMF, or cyclosporine/MMF immunosuppression. The primary endpoint of rejection (ISHLT >3A) or rejection with hemodynamic compromise demonstrated lower events in the tacrolimus/MMF group when compared to the cyclosporine/MMF group at 1 year (23.4% versus 36.8%; $p = 0.29$). Secondary end-points looking at renal function, triglyceride levels, fungal infections, and wound healing favored tacrolimus/MMF as the more advantageous immunosuppressive regimen compared to tacrolimus/sirolimus or cyclosporine/MMF (Kobashigawa et al. 2006a). These larger randomized trials substantiated the initial findings seen in the European and US experience and have solidified tacrolimus–MMF–corticosteroids as the primary

Table 1 Studies comparing tacrolimus-based regimens to cyclosporine-based regimens

Study	Type of study	Number of patients	Immunosuppressive regimens	Pertinent findings with regard to tacrolimus
(Grimm et al. 2006)	RCT	314	Tacrolimus vs. Cyclosporine + Azathioprine and Steroids	– Lower likelihood of rejection at 6 months – Lower likelihood of new-onset diabetes, dyslipidemia, hypertension
(Kobashigawa et al. 2006a)	RCT	343	Tacrolimus + Sirolimus Tacrolimus + MMF Cyclosporine + MMF (in addition to steroids)	– Lower risk of rejection at 1 year – Improved triglyceride profile and renal function
(Kobashigawa et al. 2006b)	RCT	67	Tacrolimus vs. Cyclosporine + Azathioprine and Steroids	– No difference in rejection at 5 years – Lower incidence of hypertriglyceridemia, hypertension, renal dysfunction
(Taylor et al. 1999)	RCT	85	Tacrolimus vs. Cyclosporine + Azathioprine and Steroids	– No difference in rejection – Lower incidence of hypertension, hyperlipidemia
(Meiser et al. 1998)	RCT	73	Tacrolimus vs. Cyclosporine + Azathioprine and Steroids	– No difference in rejection – Lower incidence of hypertension
(Reichart et al. 1998)	RCT	82	Tacrolimus vs. Cyclosporine + Azathioprine and Steroids	– No difference in rejection – Lower incidence of hypertension
(Rinaldi et al. 1997)	RCT	25	Tacrolimus vs. Cyclosporine + Azathioprine and Steroids	– Lower risk of rejection – Lower incidence of hypertension

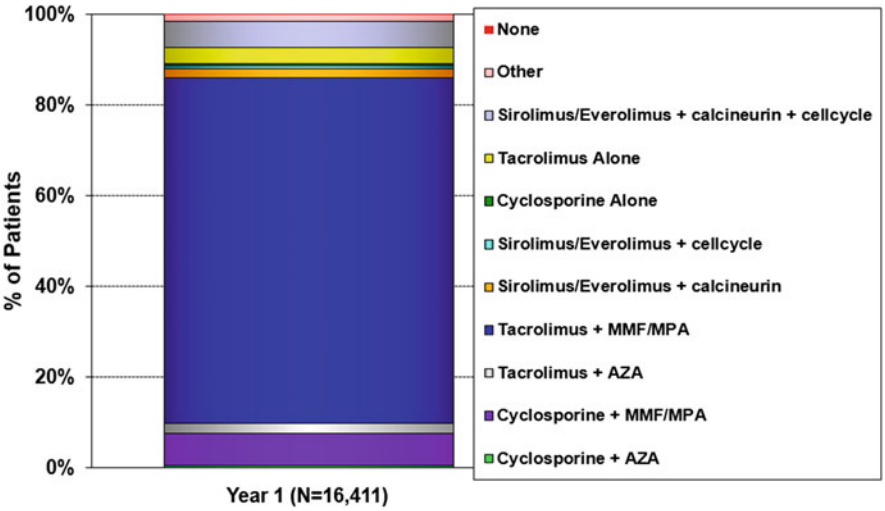

Fig. 3 ISHLT Registry of Adult Heart Transplants. Maintenance immunosuppression drug combinations at time of 1 year follow-up (Follow-ups: January 2009 to June 2017). https://ishltregistries.org/registries/slides.asp

triple-drug immunosuppressive regimen currently used by most centers worldwide (Fig. 3).

Given the success of tacrolimus-based regimens, additional trials were performed to evaluate the feasibility of tacrolimus monotherapy in cardiac transplantation. The most important of these trials was the TICTAC trial, which compared tacrolimus monotherapy to tacrolimus and MMF combination therapy (Baran et al. 2007, 2011). This trial included 150 patients who received tacrolimus, MMF, and oral steroids for the first 2 weeks post-transplant. Patients were then randomized to the MONO arm, where MMF therapy was discontinued at 14–28 days, or the COMBO arm, where MMF therapy was maintained. Freedom from Rejection ($\geq$2R rejection) was reported in 85% at 6 months and 85.9 at 12 months for the MONO arm and 94.4% at 6 months and 93% at 12 months for the COMBO arm; $p = 0.16$. Freedom from CAV (96% at 5 years for both groups; $p = 0.34$) and all-cause mortality (87.2% at 5 years for the MONO arm and 90.6% at 5 years for the COMBO arm; $p = 0.19$) were both comparable between the two groups. While the trial was underpowered to detect a difference in all-cause mortality, incidence of rejection or incidence of CAV, the overall rates of allograft rejection and CAV were low in both arms. Although very intriguing, these results need to be tempered by the fact that this was a small sample size of only 150 patients. Future data examining monotherapy with tacrolimus need to be conducted before this regimen can be considered a mainstay immunosuppressive strategy for heart transplant recipients.

Additional advancements in the field of immunosuppression were made with the advent and use of the mammalian target of rapamycin (mTOR) inhibitors, also known as proliferation signal inhibitors (PSI). The two most studied drugs in this

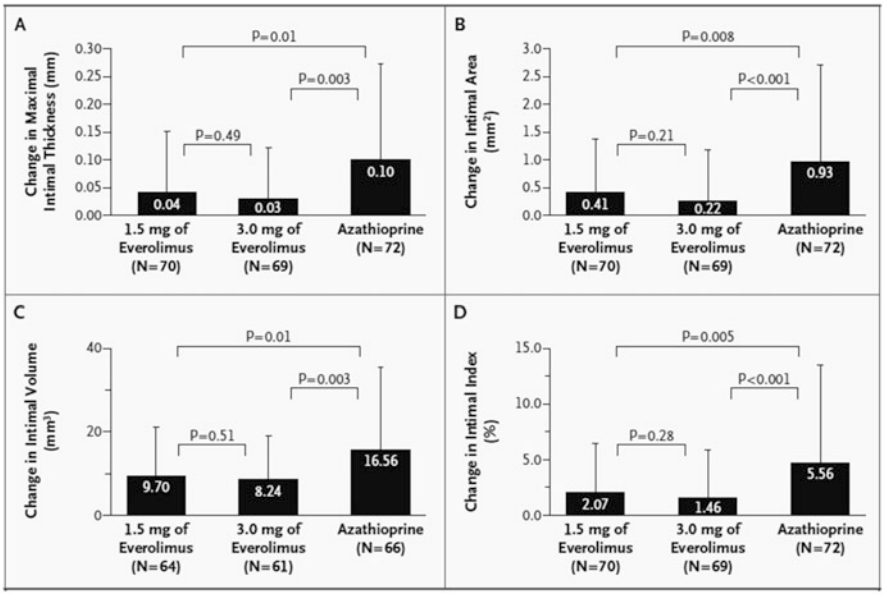

Fig. 4 Baseline to 12-Month Mean Change in Maximal Intimal Thickness (Panel A), Intimal Area (Panel B), Intimal Volume (Panel C) and Intimal Index (Panel D) in Patients Receiving Everolimus versus Azathioprine in combination with Cyclosporine and Corticosteroids (Eisen et al. 2003)

class are sirolimus and everolimus. One of the first major trials by Keogh et al. involving this class of medications compared sirolimus to azathioprine. This study randomized 136 patients in a 1:1:1 fashion to sirolimus 3 mg/day, sirolimus 5 mg/day, or azathioprine in combination with cyclosporine and steroids (Keogh et al. 2004). The study demonstrated a reduced rate of significant rejection with use of sirolimus. At 6 months, the proportion of patients with grade 3A or greater rejection was 32.4% for sirolimus 3 mg/day ($p = 0.027$), 32.8% for sirolimus 5 mg/day ($p = 0.013$), and 56.8% for azathioprine. The study also showed a reduced rate of CAV at 6 months and 2 years in the sirolimus group. A subsequent study by Eisen et al. randomized 634 patients to 1.5 mg of everolimus per day, 3 mg of everolimus per day or 1–3 mg of azathioprine per kilogram of body weight per day in combination with cyclosporine, corticosteroids, and statins (Eisen et al. 2003). The primary efficacy end point was a composite of death, graft loss or retransplantation, loss to follow-up, biopsy-proved acute rejection of grade 3A, or rejection with hemodynamic compromise. The percentage of patients that reached the primary efficacy endpoint was smaller for the 3 mg everolimus (27%, $p < 0.001$) and the 1.5 mg everolimus (36.4%, $p = 0.03$) groups when compared to patients receiving azathioprine (46.7%). In a subgroup of patients in the study receiving intravascular ultrasonography, there was a significant reduction in the development of CAV (Fig. 4). A later study evaluated everolimus in comparison with MMF with regard to survival and rejection rates. Eisen et al. randomized 721 patients to everolimus 1.5 mg or

3.0 mg with reduced-dose cyclosporine or MMF with standard-dose cyclosporine, both in combination with steroids (Eisen et al. 2013). At 12 months, there was no significant difference in the composite primary endpoint of biopsy-proven acute rejection, acute rejection associated with hemodynamic compromise, graft loss/ retransplant/ death, or loss to follow-up (35.1% in the everolimus group vs. 33.6% in the MMF group). Patients receiving everolimus, however, demonstrated a significant reduction in the rate of CAV compared to those receiving MMF (12-month increase in wall thickness 0.03 mm in everolimus group and 0.07 mm in the MMF group; $p < 0.001$). Patients receiving everolimus were noted to have a higher risk of pericardial effusion, leukopenia, anemia, and hypotension when compared to MMF. In addition, the 3 mg everolimus arm of the trial was terminated prematurely due to higher mortality.

3 Corticosteroid Weaning Protocols

Corticosteroids have been an important component of immunosuppressive regimens used in cardiac transplant recipients from the initial transplants in the late 1960s through the early 2010s. Nonetheless, given the notable adverse effects associated with prolonged corticosteroid use, investigators aimed to determine whether or not steroid-weaning and/or steroid-free regimens were feasible in heart transplant recipients. One of the first studies was conducted as early as 1985 comparing steroid weaning to low-dose steroid maintenance in a small cohort of patients (Renlund et al. 1987). This study demonstrated successful weaning of steroids after transplantation with a low cumulative incidence of rejection in that group. More importantly, the study showed that patients deemed at low risk for rejection tolerated early withdrawal (within 4 months) without any long-term consequences. This paved the way for further, larger trials involving steroid weaning.

Kobashigawa et al. conducted a similar-sized trial with a more regimented weaning protocol in patients at least 6 months following transplantation (Kobashigawa et al. 1992). In this trial, patients were monitored with endomyocardial biopsies with results guiding the weaning process. Approximately 80% of the enrolled subjects were weaned off steroids without significant rejection. Rejection episodes in subjects were largely attributable to nonadherence. A subsequent study by Olivari et al. demonstrated that steroid weaning by 6 months post-transplant was associated with no significant difference in survival, allograft function, or CAV (Olivari et al. 1995). Importantly, subjects who underwent steroid weaning were at significantly higher risk of experiencing acute rejection. A more recent study by Teuteberg et al. demonstrated that steroid withdrawal by 1 year post-transplant was feasible without increasing mortality, rejection, or CAV (Teuteberg et al. 2008). More importantly, this study compared slow steroid weaning to faster tapering and found no difference in acute rejection rates between these two methods. An additional retrospective trial investigating steroid withdrawal within 2 months post-transplant also revealed a lower risk of short- and long-term mortality and allograft rejection in the group of patients who successfully underwent weaning of

steroids (Taylor et al. 1996). This again highlights the opportunity to try to target low-risk patients undergoing cardiac transplant that benefit from early steroid weaning.

Given the data behind steroid weaning, current International Society of Heart and Lung Transplantation guidelines recommend early steroid weaning, low-dose steroid maintenance, or steroid avoidance as acceptable therapeutic approaches (Costanzo et al. 2010). The aforementioned studies conclude similar benefits and adverse consequences of steroid-free and steroid-withdrawal protocols: (1) good mid- and long-term graft/patient survival, (2) higher incidence of acute rejection in steroid-free approaches, (3) variable incidence of infection episodes, (4) lower serum cholesterol levels, (5) possibly lower hypertension rate, (6) amelioration of weight control, and (7) slightly lower risk of diabetes and bone loss. Given these recommendations and benefits, most institution-based protocols call for standard dose reductions in steroid therapy over the first 6 months post-transplant guided by endomyocardial biopsy results.

4 Induction Immunotherapy

Induction immunotherapy (IT) has been used to provide high-intensity immunosuppression in the early post-transplant period. The two most commonly utilized agents are the interleukin-2 receptor antagonists (IL2RA) and polyclonal ATG. Currently, approximately 50% of cardiac transplant programs routinely utilize induction immunosuppression, with 30% of programs utilizing IL2RA and 20% preferring ATG. The utility of induction therapy lies in the ability to delay initiation of nephrotoxic calcineurin inhibitor-based therapy in patients with renal insufficiency in the immediate post-transplant period as well as facilitation of early steroid weaning post-transplant (Cai and Terasaki 2010). Furthermore, patients at high risk for rejection such as those with preformed antibodies or highly sensitized patients and African-American individuals may derive benefit from induction therapy (Higgins et al. 2005). To date, however, studies comparing induction therapy with no induction therapy have shown no difference in efficacy.

A recent systematic review of the Cochrane Database sought to examine the effects of induction therapy versus no induction therapy and IL2RA therapy versus ATG therapy with regard to all-cause mortality, moderate-to-severe rejection, infection, and malignancy (Briasoulis et al. 2018). Included in the analysis were eight randomized-controlled trials and three observational case–control trials with a total of 1,105 patients. When comparing IT to no IT, there were no significant differences in all-cause mortality, moderate-to-severe rejection, infection, or malignancy. When comparing IL2RA therapy to ATG therapy, there were no significant differences in all-cause mortality, infection or malignancy; however, there was a notable increase in moderate-to-severe rejection in patients who received IL2RA compared to those who received ATG. These differences were primarily driven by the case–control studies, which showed a difference, while the randomized-controlled trials showed no difference in rejection rates between these two agents.

5 Immunosuppression in Selected Patient Populations

5.1 Patients with CNI-Related Renal Toxicity

Discontinuation or dose reduction of a CNI may be warranted in patients with progressive or persistent renal dysfunction. Immunosuppressive strategies for these patients include replacement of the CNI with an mTOR inhibitor (everolimus or sirolimus) or the combined use of low-dose CNI with mTOR inhibitor therapy. The beneficial effects of these strategies on renal function may be time dependent. Gude et al. showed improved GFR in patients switched to everolimus within a mean of 5.5 months of transplant compared to no improvement in a group of long-term survivors converted to everolimus at a mean of 96 months post-transplant (Gude et al. 2010). These findings, albeit demonstrated in a small patient population, suggest that the potential for renal recovery is limited following long-term CNI exposure.

The SCHEDULE trial was the first to examine the effects of early CNI withdrawal on renal function in de novo transplant patients (Andreassen et al. 2014). In this multicenter trial, 115 patients were randomized to receive MMF and corticosteroids with either conventional cyclosporine or low-dose everolimus (target level of 3–6 ng/mL) combined with low-dose cyclosporine. At 7–11 weeks post-transplant, the latter group was switched to full dose everolimus (target level 6–10 ng/mL) with removal of cyclosporine. At 1 year, mean creatinine clearance was greater in the everolimus group compared to the cyclosporine group (79.8 ± 17.7 mL/min/1.73 m^2 versus 61.5 ± 19.6 mL/min/1.73 m^2 ($p < 0.001$). Follow-up studies by the SCHEDULE investigators in a subset of the study group revealed prolonged benefits of everolimus on renal function. At 3 years, the mean difference in measured GFR between the treatment groups was 18.3 mL/min/1.73 m^2 (77.4 ± 20.2 mL/min/1.73 m^2 versus 59.2 ± 17.4 mL/min/1.73 m^2; $p < 0.001$), favoring everolimus (Fig. 5) (Andreassen et al. 2016; Nelson et al. 2017). At 5–7 years post-transplant, the mean measured GFR continued to favor everolimus (74.7 mL/min versus 62.4 mL/min) (Gustafsson et al. 2020).

Despite the observed benefits on measured GFR, mTORi therapy has been associated with the development or worsening of proteinuria. This adverse effect may be explained by the effects of mTORi on vascular endothelial growth factor resulting in increased cell membrane permeability and focal segmental glomerulosclerosis. The SCHEDULE investigators examined the development of albuminuria, a dose-related adverse effect of everolimus, in association with measured GFR at 12 and 36 months post-transplant (Nelson et al. 2017). In a relatively small subset of 66 patients, median GFR was significantly higher in the everolimus group at both 1 and 3 years post-transplant. Median urine albumin/creatinine concentration (UACR) was also significantly higher at 1 year in the everolimus group; however, there was no correlation between log UACR and measured GFR at 1 and 3 years ($r = -0.01$ and $r = 0.15$, respectively). These preliminary data derived from a small subset of patients raised questions about the clinical significance of moderate proteinuria associated with everolimus therapy.

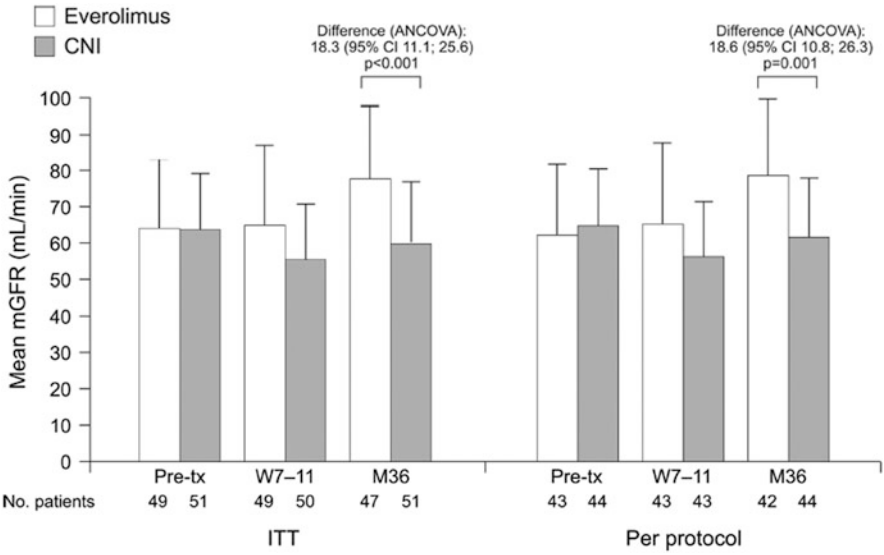

Fig. 5 Mean measured GFR before transplant and at 7–11 weeks and 36 months after transplant in patients on Calcineurin Inhibitors versus Everolimus in the SCHEDULE Study (Andreassen et al. 2016)

Most recently, investigators from the Mayo Clinic described a subset of 137 patients who were switched to sirolimus (plus antimetabolite) within a median of 1 (IQR 0.55–2.3) year post-transplant and had 24-h measurements of urine protein at time of conversion and 1 year post mTORi conversion (Asleh et al. 2020). A total of 36 patients (26%) had significant increases in urine protein from a median baseline of 235.5 (IQR 122, 460.3) mg/24 h to 1265.5 (744, 1861) mg/24 h at 1 year. Compared to the 101 patients without proteinuria, patients with proteinuria had higher baseline 24-h urine protein (235.5 versus 120.0 mg/24 h; $p = 0.004$), serum creatinine (1.8 ± 0.83 versus 1.4 ± 0.41 mg/dL; $p = 0.002$), and lower eGFR (48.8 ± 24.4 versus 63.9 ± 28.1 mL/min/1.73 m^2; $p = 0.005$). At a median follow-up of 8.6 (IQR 6–12.4) years, patients with proteinuria also had higher adjusted all-cause mortality (HR 3.8, 95% CI 1.4–1.9, $p = 0.01$). As such, the development or worsening of proteinuria within the first year of mTOR1 therapy has been described as a warning sign or harbinger of negative outcomes (Eisen 2020).

Current literature on the renal effects of mTOR inhibitory therapy, used in place of or in combination with low-dose CNI therapy in both de novo and maintenance heart transplant recipients, has been summarized by Fine and Kushwaha (Fine 2016). Comparison of published studies is hindered by a number of factors including differences in the enrolled patients (de novo or maintenance), the extent of renal impairment at baseline, measurements of renal function, CNI and mTOR inhibitor dosing methods, target serum concentrations of both the CNI and mTOR inhibitor, and the timing of follow-up assessment (6 months to 5 years). Although studies have

shown varied results between CNI and mTOR inhibitor-based regimens, the weight of the published evidence suggests that a CNI-sparing regimen with replacement by an mTOR inhibitor prevents further decline in renal function. Of note, these benefits must be balanced against the high risk of adverse reactions to mTOR inhibitors, particularly in de novo patients, including impaired wound healing, pericardial effusion, pleural effusion, and proteinuria. Discontinuation rates of mTOR inhibitor therapy, ranging from 20%–50% in clinical trials, were largely attributed to the occurrence of dose-related adverse effects including the long-term complications of stomatitis and mucocutaneous ulcers, interstitial pneumonitis, and dyslipidemia (Fine 2016). In the recently published EVERHEART study, the cumulative incidence of a safety endpoint (defined on the basis of wound healing delays, effusions needing drainage, and renal insufficiency) was compared in de novo transplant patients receiving immediate everolimus (within 144 h post heart transplant) and delayed everolimus (at 4–6 weeks post-transplant) with reduced dose cyclosporine (Potena et al. 2018). The safety endpoint was more prevalent in the immediate everolimus group (44.9%) compared to the delayed group (32.6%), supporting a safety benefit to delayed introduction of everolimus in de novo heart transplant patients. The primary driver for this difference in safety outcomes was a higher rate of pericardial effusion in the immediate everolimus group (33.7% versus 19.6%, $p = 0.04$); the rates of wound healing delays, pleural effusion, and renal insufficiency were comparable between the two groups.

5.2 Patients with Evidence of Cardiac Allograft Vasculopathy (CAV)

Early rapid intimal thickening is predictive of CAV-related adverse events and reduced survival in heart transplant recipients. Incorporation of statins into the post-transplant medication regimen has resulted in a lower incidence of CAV. In a landmark study, initiation of pravastatin within 1–2 weeks of transplant at a dose of 20 mg/day, titrated to 40 mg/day after 1 month, resulted in better survival (94% versus 78%; $p = 0.025$) and a lower incidence of CAV as determined by angiography at 1 year compared to a control group (Kobashigawa et al. 1995). In terms of immunosuppression, early conversion to everolimus or sirolimus, agents with antiproliferative and antifibrotic properties, has also been shown to limit CAV progression. Similar to studies describing the renal benefits of mTOR inhibitor therapy, studies of allograft vasculopathy support that the timing of mTOR inhibitor initiation influences the extent of benefit on CAV progression. As mentioned previously, Eisen et al. randomized patients within the first 72 h of transplant to everolimus (1.5 mg/day or 3 mg/day) or azathioprine (1–3 mg/kg/day), in combination with oral cyclosporine and corticosteroids. All patients also received statins. At 1 year, the incidence of vasculopathy in both everolimus dosing groups was significantly lower than that of the azathioprine group (Fig. 3). In contrast, in the NOCTET trial, 111 heart transplant recipients were randomized to receive either everolimus combined with low-dose CNI or standard CNI regimen at a mean of 5.8 years post-

transplant (Arora et al. 2011). At 1 year, no significant difference was observed between the two groups in CAV progression as determined on the basis of changes in maximal intimal thickness.

Early conversion to an everolimus- or sirolimus-based regimen has consistently been associated with decreased CAV progression as determined on the basis of intravascular ultrasound studies (IVUS) for extent of plaque volume, maximal intimal thickness, and plaque index (plaque volume-to-vessel volume ratio). In the SCHEDULE trial, the incidence of CAV associated with early everolimus initiation was 50% ± 7.4% in 1 year versus 64.6% ± 6.9% ($p = 0.003$) in the patients receiving a conventional cyclosporine regimen (Andreassen et al. 2014). These benefits were sustained at 36 months in a smaller subset of patients (Arora et al. 2018). Of note, everolimus's beneficial effects were observed only in those patients without underlying donor disease. Limitations of this follow-up study were the small cohort of patients with donor disease and the restricted imaging of the left ascending artery as the surrogate marker for all potential CAV.

Earlier studies comparing mycophenolate- and azathioprine-based regimens revealed significantly less progression in intimal thickening at 1 year in the mycophenolate-treated patients (Kobashigawa et al. 2006c). In a subset of the RAD001 A2310 study, mycophenolate 3 g/day plus standard-dose cyclosporine was compared to everolimus 1.5 mg/day plus reduced-dose cyclosporine in the prevention of CAV in de novo heart transplant recipients (Kobashigawa et al. 2013). In the 189 patients with evaluable IVUS data, the average change in maximal intimal thickness was significantly less at 1 year in the everolimus group (0.03 mm versus 0.07 mm, $p < 0.001$). Moreover, the incidence of CAV was 12.5% in the everolimus group versus 26.7% in the mycophenolate group ($p = 0.018$). Despite evidence to support that everolimus increases serum lipid concentrations, everolimus was shown in this study to decrease the progression of intimal thickening regardless of lipid levels. A lower frequency of CMV infection at 1 year in the everolimus group (8.2% versus 20.5%; $p < 0.001$) may have also contributed to the observed CAV benefit (Kobashigawa et al. 2013).

In the largest follow-up study to date, Asleh et al. employed a cohort of 402 heart transplant recipients to examine the long-term effectiveness of early conversion to a sirolimus-based regimen on CAV progression and outcomes (Asleh et al. 2018). Patients were converted to sirolimus ($n = 235$) due to impaired renal function, CAV detected on annual coronary angiography, or intolerance to CNI-related side effects. Conversion occurred at a median of 1.1 years (interquartile range: 0.6–3 years) after heart transplant, and doses were adjusted to maintain a target sirolimus level of 10–14 ng/mL. The comparative group ($n = 99$) were patients maintained on a CNI-based regimen (cyclosporine or tacrolimus). Patients in both the CNI and sirolimus groups also received an antimetabolite and corticosteroid. At a mean follow-up of 8.9 years from heart transplant, progression in plaque volume and plaque index was significantly lower in the sirolimus group compared to those treated with the conventional CNI-based regimen. Both all-cause mortality and CAV-related events were lower in the sirolimus-treated group (hazard ratio 0.47 [95%CI 0.31–0.7] and 0.35 [95%CI 0.21–0.59]), respectively. A subset analysis also supported more

favorable outcomes in association with early conversion to sirolimus (≤2 years from time of transplant) as opposed to late conversion (>2 years).

Before converting a patient to mTOR inhibitor therapy to lower CAV progression, consideration must be given to the aforementioned adverse effects associated with this drug class and the potential for drug discontinuation due to drug intolerance. In addition, comparative rates of rejection between mTor inhibitor and CNI-based regimens need to be considered. In the SCHEDULE follow-up trial, 41% of patients ($n = 15$) in the everolimus group had grade 2R or greater rejection at 36 months compared to 13% ($n = 5$) in the cyclosporine group ($p = 0.01$) (Arora et al. 2018). In the long-term study by Asleh et al., no significant difference was found in the incidence of hemodynamically significant rejection between the sirolimus and CNI groups (5.6% versus 9.1%; $p = 0.23$), respectively (Asleh et al. 2018).

5.3 Patients with History of Medication Nonadherence

Lack of adherence with the immunosuppressive regimen is a contributor to late rejection and graft loss in heart transplant recipients. Conversion to a once-daily extended release formulation of tacrolimus is a potential strategy to simply the immunosuppressive regimen. The pharmacokinetic profile of a once-daily extended release tacrolimus formulation (Astagraf XL) has been shown to be comparable to that of the twice-daily immediate release formulation in stable heart transplant recipients. Alloway et al. compared the steady state area under the curve ($AUC_{0–24}$) and the minimum (trough) concentration (C_{min}) achieved with the immediate and extended release formulation in 85 heart transplant recipients (81% male, 93% white, and ≥6 months after transplant) (Alloway et al. 2011). In this prospective, single arm pharmacokinetic study, patients were maintained on twice-daily tacrolimus for 2 weeks, then converted to once-daily tacrolimus on a 1:1 (mg/mg) total daily dose basis. Doses were adjusted during the 5-week study to maintain whole blood tacrolimus concentrations of 5–15 ng/mL. In the 42 (52.9%) patients who completed the five 24-h pharmacokinetic profiles, steady state $AUC_{0–24}$ and C_{min} were found to be comparable with the formulations. The mean $AUC_{0–24}$ ratio for tacrolimus once daily to twice daily was 90%, supporting a 10% lower exposure of tacrolimus with the once-daily formulation. Approximately one third of patients required dose adjustments following conversion to the once-daily formulation, the majority being dose escalations to maintain the target tacrolimus trough concentration. Mean ± SD values for serum creatinine, creatinine clearance, and plasma glucose did not differ significantly between the study phases, and there were no reported incidences of acute rejection, graft loss, or death.

Subsequent studies have been performed to identify the optimal tacrolimus dose for conversion to Astagraf XL and to assess the safety of such conversion. In 75 stable heart transplant patients, a 25% increase in dose was employed upon conversion to the Astagraf XL (Marzoa-Rivas et al. 2010). In the first 3 months following conversion, 31% of patients did not require a dosage adjustment to

maintain the target serum trough concentrations. In 68% of patients, tacrolimus concentrations remained within the target range throughout the 3 months. No significant changes were observed in blood pressure, or in plasma glucose, creatinine, and lipid concentrations following conversion to the once-daily formulation. As noted by these authors, strict monitoring of tacrolimus trough concentrations is advised during the initial months following formulation conversion to identify those patients who will require dosage adjustments.

The safety of formulation conversion has also been studied with a focus on rates of acute rejection and infection. In a retrospective study of 467 stable heart transplant patients converted to Astagraf XL, the infection rate was similar pre- and post-conversion (9.2/100 patient-years versus 10.6/100 patient-years) (Gonzalez-Vilchez et al. 2019). Six months post-conversion, five patients (1.1%) met the primary outcome of an episode of acute rejection defined by clinical suspicion, echocardiogram, or biopsy results. This study employed a 10% higher dose upon conversion to the once-daily formulation. At 2 years post-conversion, the drop-out rate was 7.1% and largely attributed to undisclosed adverse effects. Most recently, Gonzalez-Vilchez studied Astagraf XL as de novo therapy ($n = 94$) compared to initiation of standard release tacrolimus ($n = 42$) and early conversion from standard to Astagraf XL ($n = 44$) (Gonzalez-Vilchez et al. 2018). Early conversion occurred within 6 months of heart transplant (mean 4.2 ± 1.4 month). Similar rates of acute rejection, infection, and CMV infection/disease were found in the three treatment groups. The 1 year rejection rate, the primary outcome, was 1.05 (95%CI 0.51–1.54), 1.39 (95%CI, 1.00–1.78), and 1.11 (95%CI, 0.58–1.65) episodes per patient-years in the standard release, de novo, and early conversion groups, respectively. Of note, induction therapy with basiliximab, daclizumab, or OKT3 was used in 84.4% of these recipients. No significant differences were observed between the daily doses and trough tacrolimus concentrations between the groups. Limitations of this study were its retrospective design and the restricted study period of the first year post transplantation.

Use of an extended release tacrolimus formulation in heart transplant recipients is not recommended per ISHLT guidelines. Two different extended release formulations, Astagraf XL and Envarsus XR, have been approved for use following kidney transplant, and only Astagraf XL has been studied thus far in the heart transplant recipient. A phase II open label study of Envarsus XL in heart transplant recipients is currently underway with results anticipated in 2022 (ClinicalTrials.gov 2019). Of note, the pharmacokinetics of the two available extended release formulations (i.e., oral bioavailability, Tmax and Cmax) are different such that these formulations are not interchangeable. In studies of Astagraf XL, the optimal dose for conversion from the immediate to the extended release formulation was not determined; a 10–25% higher dose may be needed to facilitate maintenance of the target trough concentration. A good correlation was shown between the $AUC_{0–24}$ and the C_{min} for both Astagraf XL ($r = 0.94$) and the standard formulation ($r = 0.91$) (Marzoa-Rivas et al. 2010). These findings support use of the same therapeutic monitoring methods for both formulations (i.e., trough serum concentrations) with the same targeted trough concentrations. In stable heart transplant patients with

history of medication nonadherence, conversion to an extended release formulation is at the discretion of the prescriber. Strict therapeutic drug monitoring is recommended thereafter to ensure maintenance of target whole blood trough tacrolimus concentrations.

5.4 Patients with the Development of Cancer

Despite the improvement in survival after heart transplantation due to advancements in immunosuppressive therapy, long-term use of immunosuppression has been shown to increase the risk of malignancy. Reduced immune surveillance, drug specific properties of CNIs and the proliferation of oncogenic viruses are believed to contribute to this increased risk (Guba et al. 2004; Hojo et al. 1999). Recent data from Youn et al. examining the trends in the development of new malignancies after heart transplantation revealed 10% of patients developed cancers between 1 and 5 years after transplantation (Youn et al. 2018). The most common malignancies found were skin cancer, prostate cancer, lung cancer, and post-transplant lymphoproliferative disorders. When looking at the trends over a 5-year period between 2000–2005 versus a time period between 2006–2011 there was an increase in de novo skin cancer (6.4–8.4%; $p < 0001$) and non-skin solid cancer (4% versus 4.5%; $p = 0.004$) and no change in the incidence of lymphoproliferative disorders (1% versus 0.9%; $p = 0.118$). Importantly, survival of patients after malignancy development was much worse than for those patients without malignancy for all cancer types including skin cancer. This is consistent with prior data from the International Society for Heart and Lung Transplantation Registry supporting malignancy as the leading cause of death among long-term heart transplant survivors (Lund et al. 2014).

In direct contrast to the tumor promoting properties of calcineurin inhibitors, the mTOR inhibitors sirolimus and everolimus have been shown to have antineoplastic properties (Geissler et al. 2008). A study by Kaufman et al. in kidney transplant recipients examined the effect of maintenance immunosuppressive regimens on the development of new malignancies (Kaufman et al. 2005). The incidence rates of patients with any de novo post-transplant malignancy were 0.60% with sirolimus/everolimus alone, 0.60% with sirolimus/everolimus plus cyclosporine/tacrolimus, and 1.81% with cyclosporine/tacrolimus ($p < 0.0001$). These data suggest that mTOR inhibitors have a protective effect on the development of de novo post-transplant malignancies. More recently a multicenter, randomized open-labeled trial by Euvrard et al. assigned kidney transplant recipients who were taking a CNI and had at least one cutaneous squamous cell carcinoma to either sirolimus as a substitute for the CNI or continued initial treatment (Euvrad et al. 2012). The primary end point was survival free of squamous cell carcinoma at 2 years follow-up. A significant reduction in new squamous cell carcinomas was observed in the sirolimus group when compared to the CNI group (22% versus 39%; $p = 0.02$). Of note, in the sirolimus group, 23% of patient discontinued the drug because of adverse events. These results support that switching from a CNI to sirolimus, if tolerated, has an

antitumor effect in transplant recipients with a history of previous skin cancers. The data for heart transplantation is more limited regarding the use of mTOR inhibitors to diminish tumor development. Wang et al. retrospectively analyzed 454 heart transplant patients who received either MMF or everolimus. During a median follow-up period of 69 months, malignancy was diagnosed in a total of 27 patients receiving MMF ($n = 23$) or everolimus ($n = 4$). There was a significant difference in risk between these two groups (9.91% vs. 1.80%; $p = 0.001$). The most common malignancies were non-Hodgkin lymphoma, skin malignancy, and lung squamous cell carcinoma. The 2-year overall survival after malignancy was 50% in the everolimus group and 47% in the MMF group. One major limitation in this study was the small number of patients with malignancy despite a long follow-up period (Wang et al. 2016). Based on these and other data, most programs attempt to switch patients who develop post-transplant malignancies from a CNI to an mTOR inhibitor-based regimen.

6 Summary

Immunosuppression in heart transplantation has evolved significantly since the initial concept of immunosuppression arose. At the present time, several observations can be made from the multitude of trials that have been conducted in this field. Induction immunosuppression has not shown overall benefit versus no induction therapy, but select patient populations may benefit from its use (e.g., highly allogeneic individuals, younger patients, patients with renal dysfunction). Corticosteroid weaning has been shown to be safe within the first 6 months post-transplant and helps mitigate the risks associated with long-term steroid therapy. Maintenance immunosuppression regimens consisting of tacrolimus and mycophenolate have shown the highest level of efficacy as well as the most tolerable adverse effect profile in the general heart transplant recipient population. However, studies comparing the use of the newer proliferation signal inhibitors (sirolimus and everolimus) have shown benefit in special patient populations such as those with chronic renal dysfunction and accelerated CAV. Important areas warranting further investigation are the use of proliferation signal inhibitors in patients who develop cancer post-transplant and the use of extended release preparations of tacrolimus to improve medication adherence and tolerability. Further advances in this field will serve to maintain/improve rejection rates while reducing the risk of adverse effects as currently experienced with contemporary immunosuppression regimens.

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Immunosuppression in Lung Transplantation

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Abstract

Immunosuppression in lung transplantation is an area devoid of robust clinical data. This chapter will review the history of immunosuppression in lung transplantation. Additionally, it will evaluate the three classes of induction, maintenance, and rescue immunosuppression in detail. Induction immunosuppression in lung transplantation aims to decrease incidence of lung allograft rejection, however infectious risk must be considered when determining if induction is appropriate and which agent is most favorable. Similar to other solid organ transplant patient populations, a multi-drug approach is commonly prescribed for maintenance immunosuppression to minimize single agent drug toxicities. Emphasis of this review is placed on key medication considerations including dosing, adverse effects, and drug interactions. Clinical considerations will be reviewed per drug class given available literature. Finally, acute cellular, antibody mediated, and chronic rejection are reviewed.

Keywords

CLAD · Immunosuppression · Lung transplant

1 History of Immunosuppression in Lung Transplantation

Therapeutic advances have enabled longer allograft and patient survival following lung transplantation, with prevention of allograft recognition via immune suppression integral to success. However, lung transplantation still has worse short- and long-term outcomes compared to other solid organ groups with 5-year patient survival being 59.2% (Valapour et al. 2021). The frequency and severity of rejection episodes have been shown to be associated with increased risk of chronic lung allograft dysfunction (CLAD), in the form of bronchiolitis obliterans syndrome (BOS), and restrictive allograft dysfunction (RAS). These in turn impact survival (Estenne et al. 2002). Although evaluation of therapeutic immunosuppressive agents in lung transplantation recipients is growing, there are currently no immunosuppressants FDA-approved in lung transplantation. While necessity and data support the off-label use of various immunosuppression drugs in lung transplantation, payer-recognized drug compendia are often not up to date with clinical practice leading to difficulty in medication access in this vulnerable patient population (Lushin et al. 2021).

An increasing number of maintenance immunosuppressive agents have been introduced since the first lung transplantation procedure in 1963. Early experience was revolutionized by the approval of cyclosporine in the 1980s and its addition to

the standard regimen of azathioprine and corticosteroids. Tacrolimus replaced cyclosporine in the 1990s as a more potent inhibitor of T cell proliferation and is now recognized as the primary immunosuppressive cornerstone (Panchabhai et al. 2018). Mycophenolic acid formulations were approved in the early 2000s and have generally replaced azathioprine as the antimetabolite of choice. Mammalian target of rapamycin inhibitors (mTOR), sirolimus, was approved in the late 1990s and everolimus in 2010 for rejection prophylaxis. The co-stimulation blocker, belatacept, was the most recently introduced option in 2011. Despite advancements in immunosuppression, rejection and survival rates after lung transplantation remain suboptimal (Valapour et al. 2021).

2 Induction Immunosuppression in Lung Transplantation

Induction therapy in transplantation is intense immunosuppression, administered peri-operatively, with the goal to prevent acute rejection within the early post-transplant period. Therapies used are antibody preparations targeted at T-lymphocytes and are either lymphocyte depleting or non-depleting. The use of induction has also been recognized as a method to minimize maintenance immunosuppression, thereby limiting adverse effects associated with lifelong therapy. While the use of induction in lung transplantation has increased over the past 10 years, the clinical efficacy and the optimal agent remain debated.

2.1 Anti-thymocyte Globulin

Anti-thymocyte globulins are polyclonal anti-human IgG antibody preparations targeted at human thymocytes and lead to modulation of T cell activation and depletion from circulation via complement-dependent lysis and activation induced apoptosis (Thymoglobulin Package Insert 2017; Wiseman 2016). Anti-thymocyte globulin is derived from either rabbit (rATG, Thymoglobulin®) or horse (eATG, ATGAM®), but only rATG is approved for rejection prophylaxis in renal transplant recipients. Neither preparation is approved in lung transplant recipients. Dosing strategies for anti-thymocyte globulin are weight-based and vary by formulation. Administered as an intravenous infusion, rATG is dosed 1–1.5 mg/kg daily for 4–7 days, while eATG is 10–15 mg/kg/dose for 4–7 days (Thymoglobulin Package Insert 2017; ATGAM Package Insert 2021). In clinical practice, rATG is preferred over eATG as studies in kidney transplant recipients demonstrated significantly less rejection (Brennan et al. 1999). Both formulations can cause infusion-related reactions (fevers, chills, rigors, and arthralgia). Thus, premedication with corticosteroids, acetaminophen, and/or an antihistamine is recommended (Thymoglobulin Package Insert 2017). Leukopenia and thrombocytopenia may occur, with manufacturer suggested dosing adjustments based on the severity available for guidance.

2.2 Alemtuzumab

Alemtuzumab (Campath-1H®) is a humanized rat monoclonal antibody that targets CD52, which is present on T- and B-lymphocytes, as well as other cells of the innate immune pathway. It causes depletion of T cells via multiple proposed mechanisms including prevention of T cell co-stimulation as well as complement and antibody-dependent cytotoxicity (Wiseman 2016; van der Zwan et al. 2018). Use of alemtuzumab as induction in all solid organ transplantation is off label. Alemtuzumab is administered intravenously as a single dose of 30 mg at time of transplantation. Infusion-related reactions are possible, with premedication recommended. Given alemtuzumab's disposition to cause profound depletion of immune cells, cytopenia can be an adverse effect of its use (Campath Package Insert 2020).

2.3 Basiliximab

Basiliximab (Simulect®) is a murine/human chimeric monoclonal antibody that acts as antagonist of the interleukin-2 (IL-2) receptor alpha chain on activated T cells, limiting the signal for their proliferation and contribution to allograft rejection (Wiseman 2016). Daclizumab (Zinbryta®) has been utilized in studies of lung transplantation recipients, but was withdrawn from the US market in 2018 (US Food and Drug Administration 2018). Basiliximab, a non-lymphocyte-depleting induction agent, is administered via 20 mg intravenous infusion on post-operative day zero and four. Hypersensitivity reactions are rare, and other severe adverse related effects are similar to placebo (Simulect Package Insert 2014).

2.4 Considerations for Induction Immunosuppression in Lung Transplantation

When selecting an induction agent for lung transplantation, it should be noted that not all induction is created equal. Lymphocyte-depleting therapies, such as anti-thymocyte globulins and alemtuzumab, are highly immunosuppressive. They cause extended lymphocyte depletion ranging from 6 to 12 months depending on the cell-line (Thymoglobulin Package Insert 2017; Campath Package Insert 2020). Due to basiliximab's non-lymphocyte depleting nature its duration of action is limited to the time it saturates the IL-2 receptor, which ranges from approximately 1–2 months (Simulect Package Insert 2014).

Available data has found induction immunosuppression reduces incidence of acute cellular rejection (ACR) following lung transplantation. However, data are confounding and the impact on BOS and long-term outcomes is less apparent. Early, small retrospective studies comparing no induction to daclizumab or basiliximab showed a trend towards less rejection with use (Garrity Jr. et al. 2001; Borro et al. 2005a). A prospective study of 44 recipients evaluating the use of rATG compared to

no induction demonstrated significantly less episodes of grade >2 rejection within 6 months of transplant with rATG, without impact on eight-year graft survival (Hartwig et al. 2008). Additionally, a descriptive analysis of a randomized trial of 221 recipients did not show a difference in efficacy failure at 12 months between ATG and placebo (Snell et al. 2014).

Comparative studies of outcomes with use of IL-2RA versus ATG have also shown conflicting results. Multiple retrospective analyses have shown a lower incidence of rejection in those receiving ATG, but this has not consistently been demonstrated in RCTs (Hachem et al. 2005; Lischke et al. 2007). Concerns about risk of infections with T-cell depleting therapies have also been explored. In a small retrospective study, cytomegalovirus (CMV) was more common with rATG, particularly in CMV seronegative recipients (Clinckart et al. 2009). A prospective controlled study also found a higher rate of infections at 1 year post-transplant with T-cell depleting induction agents compared to daclizumab (Brock et al. 2001). While infection is of particular concern in cystic fibrosis (CF), some data support a survival benefit with use of induction in CF patients undergoing lung transplantation (Jaksch et al. 2013; Kirkby et al. 2015).

The use of alemtuzumab has been investigated in a small number of lung transplant studies. In an open-label, randomized study of alemtuzumab with reduced dose maintenance immunosuppression compared to rATG, a lower rate of grade ≥ 2 or higher rejection was observed with alemtuzumab (Jaksch et al. 2014). A similar trend of less rejection at 6 months was noted in those receiving alemtuzumab compared to daclizumab (McCurry et al. 2005) and a cohort who had received basiliximab (Whited et al. 2015). A retrospective analysis that investigated long-term outcomes in 336 lung transplantation recipients found greater five-year freedom from patient and allograft survival, cellular rejection, and BOS in those who received alemtuzumab relative to rATG, daclizumab, or no induction (Shyu et al. 2011).

Larger scale registry-based data have also described variable evidence. A retrospective cohort study of the International Society for Heart and Lung Transplantation (ISHLT) registry of almost 4,000 lung transplantation recipients evaluated outcomes based on type of induction. Four-year allograft survival was higher in those who received IL2-RA based induction (64%), compared to ATG (60%) or no induction (57%). Although induction immunosuppression had less cellular rejection early post-transplant, treatment for infection was higher (Hachem et al. 2008). The latest SRTR data reports one-year acute rejection rates of 15.1% in those who received IL-2RA, compared with 18.9% in those who received lymphocyte-depleting agents and 17.8% in those with no induction (Valapour et al. 2021). A Cochrane review that analyzed mortality, acute rejection, and adverse events in six randomized-controlled trials with 278 patients found no clear benefit or harm among use of various induction immunosuppression agents when compared to no induction (Penninga et al. 2013a).

The approach to use of induction immunosuppression in lung transplantation remains heterogeneous. Use may decrease the incidence of acute rejection, but its impact on long-term outcomes of BOS and allograft survival are less clear. Consensus on the strategy has not been reached and the approach is varied among transplant

institutions. Despite this, >80% of transplant centers internationally use induction immunosuppressive at the time of transplant (Chambers et al. 2019). As of 2018, IL-2RA was used in 69% of adult lung transplantation recipients with no induction was given in 22% of cases followed by T-cell depleting therapies (9%) (Valapour et al. 2018, 2021). Concerns regarding impact of T-cell targeted therapies on infectious and malignant outcomes after lung transplantation may drive clinical decisions regarding induction and patient-specific factors should be considered.

3 Maintenance Immunosuppression in Lung Transplantation

Maintenance immunosuppression is the chronic therapy patients take indefinitely to consistently suppress their immune system, thus limiting the development of acute and chronic rejection. Immunosuppression weighs efficacy for prevention of rejection against risk of infection, malignancy, and medication toxicities. However, given the complex nature of lung transplant recipients, emphasis should be made on considering patient-specific factors when deciding maintenance immunosuppression. Although the optimal immunosuppression therapy in lung transplantation is not defined, it is a multi-drug approach to preserve allograft function while minimizing medication toxicities. The most common initial maintenance immunosuppression regimen used in lung transplantation in the USA is tacrolimus, mycophenolate mofetil, and corticosteroids being used in >80% of recipients (Valapour et al. 2021). Class options include calcineurin inhibitors, DNA synthesis inhibitors, mTOR inhibitors, corticosteroids, and a co-stimulation inhibitor.

3.1 Calcineurin Inhibitors

Calcineurin inhibitors exert their activity on CD4+ T cells by preventing translocation of nuclear factor of activated T-lymphocyte (NFAT) through the nuclear membrane to its site of action. This prevents the transcription of pro-inflammatory cytokines such as IL-2, 3, 4 as well as TNF-α and IFN-γ. The overall effect results in the inhibition of T cell activation and proliferation. Notably, calcineurin inhibitors bind to different immunophilins. Cyclosporine binds to cyclophilin whereas tacrolimus binds to FK-binding protein-12 (Shibasaki et al. 2002).

Calcineurin inhibitors have a variety of side effects (Table 1). Notably, these agents can cause dose-dependent nephrotoxicity through vasoconstriction of the afferent and efferent arterioles leading to decreased renal blood flow and GFR (Textor et al. 1993). Nephrotoxic adverse effects manifest as oliguria, increases in serum creatinine, and electrolyte abnormalities. Chronic calcineurin inhibitor exposure can lead to irreversible kidney damage and acute kidney injury early after transplantation and is associated with an increased risk of chronic renal failure development (50% at 1 year and 70% at 5 years) (Paradela de la Morena et al. 2010). Also notable are their neurotoxic adverse effects. These symptoms are

Table 1 Common adverse effects of maintenance immunosuppression

	Cytopenia	Nephrotoxicity	Neurotoxicity	Hypertension	Hyperglycemia	Hyperlipidemia	Gastrointestinal	Dermatologic	Mood/ Mentation	Hepatotoxicity	Hirsutism	Osteoporosis	Edema	Ocular
Cyclosporine	X	X	X	X		X				X	X			
Tacrolimus	X	X	X	X	X					X				
Mycophenolic acids	X						X							
Azathioprine	X						X							
Corticosteroids				X	X	X	X	X	X		X	X	X	X
Sirolimus	X	[a]				X	X	X						
Everolimus	X	[a]				X	X	X					X	
Belatacept	X						X						X	

[a]Nephrotoxicity may be enhanced if given concomitantly with a calcineurin inhibitor

Table 2 Common effectors of CYP 3A4

Inhibitors (increase immunosuppression exposure)	Inducers (decrease immunosuppression exposure)
Macrolides • Erythromycin • Clarithromycin Azole antifungals • Ketoconazole • Voriconazole • Posaconazole • Isavuconazole • Itraconazole • Fluconazole Non-dihydropyridine calcium channel blockers • Verapamil • Diltiazem Protease inhibitors and boosters • Ritonavir • Cobicistat Other antiarrhythmics • Amiodarone • Dronedarone Grapefruit juice	Anti-epileptics • Fosphenytoin • Phenytoin • Carbamazepine Rifamycins • Rifampin • Rifabutin Barbiturates • Phenobarbital • Primidone • Pentobarbital Herbal • St. John's Wort

associated with the peak exposure of the medication, often manifesting 1–2 h after ingestion. They include bilateral tremor, headache, stutter, neuropathy, and seizure in severe cases. In rare situations, posterior reversible encephalopathy syndrome (PRES) may occur through endothelial injury that causes subcortical vasogenic brain edema. Also common are metabolic adverse effects including hyperglycemia and hypertension (Mayer et al. 1997). Hyperglycemia is caused by islet cell toxicity. Hypertension is caused by sodium and subsequent water retention. Hyperlipidemia is only associated with cyclosporine, is dose dependent, and is thought to occur through the inhibition of LDL cholesterol binding to the LDL receptor resulting in a decline in LDL clearance (Agarwal and Prasad 2016).

As calcineurin inhibitors are predominantly metabolized through CYP 3A4, numerous drug interactions exist. CYP 3A4 inducers will increase metabolism of agent to inactive metabolites, thus decreasing levels whereas inhibitors will have the opposite effect. A full list of CYP 3A4 inducers and inhibitors can be found in Table 2. Interestingly, cyclosporine is a weak inhibitor of CYP 3A4 and can also inhibit metabolism of weaker substrate such as HMG-COA reductase inhibitors. Thereby, increasing medication exposures increases the risk for myopathy. Specifically, simvastatin, pitavastatin, and fluvastatin should avoid being given concomitantly with cyclosporine given the effect of the drug interaction (Gengraf Package Insert 2021; Sandimmune Package Insert 2020).

The earliest calcineurin inhibitor, cyclosporine has two formulations: modified (Neoral® or Gengraf®) and USP (Sandimmune®). Dosage formulations are not interchangeable as the original USP formulation is made in a castor oil base,

exhibiting poor oral bioavailability of ~30% and high intra-patient variability in absorption due to its dependence on bile acid salts (Sandimmune Package Insert 2020). The newer, modified formulation is a microemulsion resulting in increased bioavailability and decreased intra-patient variability in absorption (Kahan et al. 1995). Given the benefits with the modified formulation, USP formulation is no longer commonly used in clinical practice.

Starting doses of oral cyclosporine range from 4 to 9 mg/kg/dose and it is dosed every 12 h. If a patient is unable to tolerate oral medication, cyclosporine can be given intravenously although this route is associated with adverse events and is challenging to monitor. If a patient is transitioned from IV: PO the dose should be decreased to 1/3 of the oral daily dose (IBM Micromedex® DRUGDEX® (electronic version)) n.d.). Food decreases the modified formulation's AUC by 13%, so patients should be educated to consistently take medications with or without food (Gengraf Package Insert 2021). There are two limited sampling strategies that can be used for cyclosporine therapeutic drug monitoring. 12-h troughs of cyclosporine (modified) had a strong correlation to AUC (Kahan et al. 1995). Two-hour peak concentration of cyclosporine is the most accurate representation of AUC and associated with clinical outcomes. (Knight and Morris 2007) However, they are challenging to obtain in clinical practice given a small 10–15 min "window of opportunity" before and after the two-hour time point of which a drawn sample will be accurate (Levy et al. 2002; Saint-Marcoux et al. 2003).

The more commonly used calcineurin inhibitor, tacrolimus, has immediate-release (Prograf®) and extended-release formulations (Astagraf® and Envarsus®). Dosage formulations are not interchangeable as LCP-tacrolimus (Envarsus®) has an increased exposure, as well as a delayed and blunted peak (Tremblay et al. 2017). Starting doses of oral tacrolimus are 0.05–0.2 mg/kg/dose and the immediate-release formulation is dosed every 12 h (Astagraf XL Package Insert 2019; Envarsus XR Package Insert 2018; Prograf Package Insert 2018). If a patient is unable to tolerate oral medication, immediate-release tacrolimus can be administered sublingually by opening the capsule and administering the capsule contents under the patient's tongue. PPE should be used if opening capsules (Doligalski et al. 2014). The conversion from PO:SL immediate-release tacrolimus ranges from 2:1 to 1:1, especially in the lung transplantation patient population (Doligalski et al. 2014; Al Sagheer and Enderby 2019). If a patient cannot take sublingual medication, an intravenous formulation exists but should be used as a last resort. Dose conversion from PO:IV is 3:1 and it should be run as a continuous 24-h infusion instead of intermittently given lower incidence of neuro- and nephrotoxicity (Abu-Elmagd et al. 1991). Food also decreases overall medication exposure by ~25% so patients should be educated to consistently take medications with or without food (Bekersky et al. 2001). 12-h troughs levels have been well correlated to overall AUC (Prograf Package Insert 2018). Cystic fibrosis patients have higher clearance of tacrolimus and may warrant higher doses and closer therapeutic drug monitoring (Knoop et al. 2005).

The best available evidence in lung transplantation has found tacrolimus to be associated with decreased rates of BOS, acute rejection, and allograft survival when

compared to either cyclosporine formulation. These benefits need to be weighed with the significantly higher rates of diabetes (Treede et al. 2001; Keenan et al. 1995). A meta-analysis was able to confirm that tacrolimus has significantly lower rates of acute rejection but was unable to find significance with rates of BOS and allograft survival. Rate of diabetes was also significantly higher while rates of renal dysfunction, hypertension, and infections did not significantly differ (Fan et al. 2009). The largest systematic review to date found tacrolimus-treated patients to have significantly lower rates of BOS while rates of acute rejection, mortality, infections and adverse effects did not differ significantly (Penninga et al. 2013b).

3.2 DNA Synthesis Inhibitors

These agents prevent lymphocyte proliferation in different ways. Mycophenolic acid formulations inhibit inosine monophosphate dehydrogenase, inhibiting de novo purine synthesis. By preventing the production of these nucleotides, mycophenolic acid disproportionately affects lymphocyte proliferation as they do not have the salvage pathway to create guanosine like other cell lines do (Allison and Eugui 1996). Azathioprine works by incorporating its metabolites, 6-thioguanine, 6-methyl-MP, and 6-thiouric acid, into DNA subsequently blocking synthesis. Lastly, the disruption of both the de novo and salvage pathways of nucleic acid synthesis conveys a more extensive and severe adverse effect profile (Imuran Package Insert 2018).

Two formulations of mycophenolic acid exist, the pro-drug (mycophenolate mofetil, CellCept®) and the enteric-coated formulation (mycophenolate sodium, Myfortic®). Of note, 720 mg of mycophenolate sodium is equivalent to 1,000 mg of mycophenolate mofetil. Mycophenolate mofetil has solid and liquid oral formulations as well as an intravenous option. Mycophenolate sodium only has enteric-coated tablets available. In patients unable to tolerate oral formulations, intravenous mycophenolate mofetil can be used and dose adjustment is 1:1 (IBM Micromedex® DRUGDEX® (electronic version) n.d.).

As these agents affect cell lines with rapid turnover, mycophenolic acid and azathioprine are commonly associated with gastrointestinal adverse effects such as nausea, vomiting, and diarrhea. Effects are thought to be dose related and associated with the glucuronidated metabolite that requires renal clearance. If possible, lower doses of all agents or thrice daily dosing of mycophenolate mofetil can be tried to assist with adverse effects. Mycophenolate sodium was originally formulated with the thought to help with gastrointestinal adverse effects, but rates were non-inferior to mycophenolate mofetil when studied (Behrend and Braun 2005). These agents also cause myelosuppression which may require dose decreases. Lastly, mycophenolic acid is teratogenic. Thus, any female of childbearing potential is required to be enrolled in the mycophenolate Risk Evaluation Mitigation Strategy program (Kim et al. 2013). Azathioprine has also been known to cause alopecia, pancreatitis, and hepatotoxicity (Imuran Package Insert 2018).

Anything that competes with tubular secretion (i.e., acyclovir) of the glucuronidated metabolite will cause retention leading to increased enterohepatic recirculation and subsequent increased medication exposure. Cyclosporine also inhibits MDR transport protein, preventing enterohepatic recirculation of mycophenolic acid, thus decreasing AUC exposure by ~30% (Cox and Ensom 2003). Also, co-administration of aluminum and magnesium- containing medications can cause an overall decreased absorption of mycophenolic acid, however clinical relevance is not yet known (IBM Micromedex® DRUGDEX® (electronic version) n.d.). Azathioprine is converted by xanthine oxidase to 6-thiouric acid to be renally eliminated. Thus, xanthine oxidase inhibitors such as allopurinol and febuxostat increase azathioprine exposure. Azathioprine doses should be decreased 50–75% if a xanthine oxidase inhibitor is used (Imuran Package Insert 2018).

Mycophenolate mofetil is dosed at 1,000 mg twice daily in patient whereas mycophenolate sodium is 720 mg oral twice daily. Plasma concentrations of mycophenolic acid can be measured, although clinical utility is unknown given cost and lack of correlation with clinical adverse effects. Mycophenolic acid AUCs $<$30 mcg/mL/h have been correlated with increased rates of rejection whereas levels $>$60 mcg/mL/h have been linked to increased leukopenia (Shaw et al. 2001; Oellerich et al. 2000). Although less frequently used in clinical practice, azathioprine is an alternative option to mycophenolic acid. Azathioprine is dosed 3–5 mg/kg daily (Imuran Package Insert 2018). Thiopurine *S*-methyltransferase (TPMT) activity can affect drug exposure and subsequent myelosuppression. Approximately 10% of the population has a polymorphism in TPMT leading to low level activity. However, routine monitoring for the TPMT polymorphism in transplantation is not currently recommended due to lack of data.

Either mycophenolate mofetil or azathioprine can be used in combination with tacrolimus or cyclosporine. Acute rejection, BOS, and patient survival were not significantly different with cyclosporine in combination with mycophenolate mofetil versus tacrolimus in combination with azathioprine in the setting of corticosteroids and IL-2 receptor antagonist induction (Glanville et al. 2015; McNeil et al. 2006; Palmer et al. 2001). However, mycophenolate mofetil may be associated with less acute rejection as compared to azathioprine in lung transplant recipients (Speich et al. 2010). Patients who do not tolerate mycophenolate mofetil may subsequently tolerate azathioprine and vice versa. Leukopenia requiring dose reduction is similar between agents but drug discontinuation for gastrointestinal adverse effects is more common with mycophenolate mofetil.

3.3 Corticosteroids

Corticosteroids have global immunosuppressive and anti-inflammatory effects by binding to glucocorticoid receptor and causing decreased production of pro-inflammatory and immune activating cytokines by interfering with gene transcription. Corticosteroids also inhibit lymphokines and cause sequestration of

T-lymphocytes in the reticuloendothelial system (Barshes et al. 2004; Frey and Frey 1990).

The most common adverse effects are metabolic including hypertension and hyperglycemia. Corticosteroids can have neurologic effects including insomnia, mood changes, irritability, mania, and even hallucinations at high doses, depression if prolonged exposure, and anxiety. They can also cause increased appetite, weight gain, edema due to mineralocorticoid fluid retention, bruising, acne, delayed wound healing, and hirsutism. Prolonged exposure can increase risk of osteoporosis, avascular necrosis of the femoral head, glaucoma, cataracts, and esophagitis (Barshes et al. 2004). Current maintenance immunosuppressive strategies seek to limit or remove corticosteroids from the multi-drug regimen although available data does not favor corticosteroid withdrawal at this time given mixed findings (Borro et al. 2005b; Shitrit et al. 2005).

A multitude of corticosteroid formulations exist including intravenous methylprednisolone, dexamethasone, and hydrocortisone as well as oral prednisone, prednisolone, hydrocortisone, and dexamethasone. Dosage formulations are not equivalent and have varying glucocorticoid and mineralocorticoid potency. Choice of agent and dosing regimens vary by institution, but generally involve high-dose intravenous formulations (usually methylprednisolone 500–1,000 mg daily) in the intra- and peri-operative period that are gradually tapered to a low-dose oral formulation (usually prednisone). Low-dose oral formulations are usually continued indefinitely although some data are supportive of withdrawal in stable patients many years post-transplantation (Borro et al. 2005b).

3.4 mTOR Inhibitors

These agents inhibit cellular response to cytokines (specifically, IL-2, 4, and 15) preventing progression of the cell cycle from G1 to S phase. The overall result is an inhibition of T cell proliferation (Rapamune Package Insert 2021). The most common adverse effect is myelotoxicity and is dose dependent. Specifically, leukopenia and thrombocytopenia have been associated with sirolimus trough concentrations >15 ng/mL (Kahan et al. 2000). Also notable, these agents commonly cause dyslipidemia. Often, medical management can be used to address hypertriglyceridemia or hyperlipidemia. Since these agents inhibit fibroblast growth factors paramount in tissue repair, they have been associated with delayed wound healing. These agents are also associated with aphthous ulcers, thought to be caused by their inhibition of epithelial growth factors. Both agents are linked to enhanced nephrotoxicity if co-administered with calcineurin inhibitors (Wiseman et al. 2013). A rare but serious adverse effect of sirolimus reported in the literature is interstitial pneumonitis that presents as low-grade fever, dyspnea on exertion, fatigue, and dry cough. It is reversed by stopping the offending agent (Weiner et al. 2007). These agents should be avoided in the immediate post-transplant setting given rare but serious anastomotic airway dehiscence (King-Biggs et al. 2003). Everolimus has

been associated with peripheral edema, constipation, and urinary tract infections (Zortress Package Insert 2021).

Similarly, to calcineurin inhibitors, mTOR inhibitors are predominantly metabolized through CYP 3A4. Thus, CYP 3A4 inducers will also increase metabolism of agent to inactive metabolites and thereby decrease levels. Whereas inhibitors will also have the opposite effect (Rapamune Package Insert 2021; Zortress Package Insert 2021). Please refer to Table 2 for all relevant inducers and inhibitors.

Sirolimus has only liquid and solid dosage forms. They are interchangeable with one another. Given its long half-life, it is recommended to load sirolimus with 6 mg followed by 2–5 mg daily dose. Food decreases sirolimus absorption by ~25% so patients should be educated to consistently take medications with or without food. Troughs are routinely used to guarantee efficacy while minimizing toxicity. Everolimus has dosage options for oncologic (Afinitor®) and solid organ transplant (Zortress®) indications, so it is paramount to confirm correct formulation given the 10-fold difference in recommended dosing. Everolimus has only solid dosage forms commercially available although liquid formulations can be compounded. A standard dose of 0.75 mg twice daily is recommended. Troughs can also be used for therapeutic drug monitoring.

Everolimus or sirolimus can be used in place of DNA synthesis inhibitors and is associated with less cytomegalovirus infection and progression to BOS (Glanville et al. 2015; Bhorade et al. 2011; Strueber et al. 2016; Sacher et al. 2014). mTOR inhibitors may not be as well tolerated, as there has been a high incidence of withdrawal (up to 64%) in studies due to drug related adverse events including impaired wound healing, thrombotic microangiopathy, and venous thromboembolism (Bhorade et al. 2011). mTOR inhibitors can also be used as a replacement to or in combination with low-dose calcineurin inhibitors for stable recipients experiencing renal dysfunction (Gottlieb et al. 2019; Gullestad et al. 2016; Roman et al. 2011). However, risks of acute rejection and adverse effects need to be balanced with minimization of calcineurin inhibitor associated nephrotoxicity.

3.5 Belatacept

By binding to CD 80 and 86, belatacept blocks co-stimulation of T-lymphocytes which are integral to the development of rejection via the afferent limb of the adaptive immune system. Inhibition prevents T-lymphocyte proliferation and production of interleukin-2, interleukin-4, interferon-γ, and TNF-α. Belatacept is available as an IV infusion that can be given peripherally over 30 min. Dosing is based on actual body weight and stays the same unless there is >10% change from baseline weight (Nulojix Package Insert 2014). The optimal dosing for belatacept in lung transplantation has not been well defined. Available literature has investigated FDA-labeled dosing of 10 mg/kg on days 1, 5, 15, 29, 45, and 59, followed by 5 mg/kg monthly thereafter as well as alternative dosing strategies including 5 mg/kg every 2 weeks for the first six doses followed by 5 mg/kg monthly as well as 10 mg/

kg on day 0, 4, 14, and 28 followed by 10 mg/kg monthly (Benninger 2021; Hui et al. 2014; Iasella et al. 2018; Timofte et al. 2016).

Side effects are generally mild and include anemia, gastrointestinal adverse effects, urinary tract infection, edema, and pyrexia, leukopenia, and potassium abnormalities. CNS PTLD, PML, and other CNS infections were more frequently observed in association with a higher cumulative and more frequent dosing regimen compared to what is FDA approved. Also, PTLD rates were higher in EBV seronegative individuals. Thus, use in EBV seronegative individuals is contraindicated (Nulojix Package Insert 2014).

In lung transplantation, data for use are currently limited. It includes a case report of a 56-year-old male bilateral lung transplant recipient with HUS, attributed to both tacrolimus and sirolimus, who was converted to belatacept, mycophenolate, and prednisone. The patient had no ACR at 6 months post-conversion (Hui et al. 2014). A case series of eight recipients with renal dysfunction on their existing calcineurin inhibitor-based regimen had belatacept added to their regimen a median of 1.5 years post-transplant to allow for temporary discontinuation or withdrawal of calcineurin inhibitors. One patient had mild ACR and patients' FEV_1 remained stable 6 months post-conversion (Timofte et al. 2016). Similar results were observed in a case series of nine lung transplantation recipients who underwent conversion. This study additionally found a statistically significant increase in mean eGFR after conversion to belatacept (Iasella et al. 2018). In the largest evaluation to date, 85 recipients were prospectively evaluated after conversion from CNI to belatacept within 1 year post-transplant. Renal function remained stable throughout conversion and a statistically significant increase in FEV_1 was observed. Belatacept was discontinued in 33% of patients evaluated, mostly due to infectious complications (Benninger 2021). A pilot randomized-controlled trial is currently underway to evaluate de novo belatacept use with CNI withdrawal compared to standard of care immunosuppression after lung transplantation (Hachem 2018–2021).

4 Rescue Immunosuppression for Lung Transplantation

4.1 Acute Cellular Rejection

Approximately 16% of lung transplantation recipients will experience ACR within the first year of transplantation, with higher rates in younger patients <50 years of age (Valapour et al. 2021). Symptoms of ACR are relatively nonspecific (cough, low grade fever, FEV_1 decline, pleural effusion, and hypoxemia). Because of this, most lung transplantation centers in the USA employ protocol surveillance bronchoscopies with transbronchial biopsies early post-transplant, but practice may vary. Additional bronchoscopies may be requested if there is a clinical suspicion of allograft rejection and may be valuable to rule out other post-transplant complications (i.e., infection). ISHLT provided pathologic grading for pulmonary allograft acute rejection as seen in Table 3 (Stewart et al. 2007).

Table 3 Adopted from Roux et al. (2019)

Treatment	Effect	Onset of treatment	Duration of treatment
Immunoadsorption	Reduction/clearance of DSA	Immediate	Few weeks
Plasmapheresis	Reduction/clearance of DSA	Immediate	Few weeks
Intravenous immunoglobulin	Decrease DSA complement binding	Days	3–4 weeks
Anti-CD20 antibodies	Reduction of DSA by depleting plasma cell progenitors	Immediate B-cell depletion Months for DSA reduction	Months
Proteasome inhibitors	Reduction of DSA by depleting plasma cells	Immediate plasma cell depletion	Months
Complement inhibitors	Decrease DSA complement binding	Days	Weeks

The gold standard of care for treatment of ACR is pulse glucocorticoids, despite limited studies there is a lack of evidence to elucidate the optimal duration and dose of corticosteroids (Yousef 2014). Depending on the severity of rejection, the dose ranges from 1 mg/kg oral prednisone up to 10–15 mg/kg intravenous methylprednisolone for higher grade rejections. After corticosteroid pulse therapy is complete, an oral prednisone taper is typically employed with a reduction in dose every 3–7 days, until the baseline maintenance dose (~5–10 mg/day) is achieved and continued indefinitely. Adverse effects associated with acute, high-dose corticosteroids may include hyperglycemia, insomnia, mood changes, mania, esophagitis, and edema. In addition to corticosteroid therapy, augmentation of current maintenance immunosuppression may be clinically indicated (Ensor et al. 2018; Sarahrudi et al. 2004). Additionally, optimizing maintenance immunosuppression, addressing nonadherence, and assessing for noncompliance are paramount to mitigate further recurrence of ACR.

Follow-up transbronchial biopsy, imaging, and pulmonary function tests are often done to confirm treatment effectiveness and ensure absence of rejection progression a few weeks post therapy. (Clelland et al. 1990) For higher grade and/or refractory ACR there is no reported consensus of therapy. However, lymphocyte-depleting therapies including rATG or alemtuzumab can be considered in well-selected patients (Ensor et al. 2017a; Reams et al. 2002). With lymphocyte-depleting therapies, addition of antiviral and antifungal prophylactic coverage is encouraged. The frequency and severity of ACR occurrences post-transplantation increase the risk for CLAD. Therefore, institutional protocols on frequent ACR assessment, especially early post-transplant, should be in place (Burton et al. 2007; Heng et al. 1998).

4.2 Antibody-Mediated Rejection

AMR in lung transplantation is an evolving and difficult to manage disease state. AMR results in increased morbidity, mortality, and health care cost (Levine et al. 2016). Furthermore, the diagnosis of AMR is evolving and currently consists of clinical or subclinical rejection with varying degrees of certainty and severity (Fig. 1) (Levine et al. 2016). The true incidence of AMR is still being elucidated, and the creation of recent guidelines will shed light on the actual impact of this disease in lung transplantation. AMR is mediated through recipient antibody recognition of donor HLA, and activation of the immune system, such as complement, and other non-complement-dependent pathways of cellular injury (Levine et al. 2016). Revealing the exact mechanisms of cellular injury secondary to antibody-mediated immune activation is a rapidly advancing and every-changing topic within transplantation.

Robust, randomized-controlled trials on AMR treatment are lacking, and much of the data published is prior to consensus guidelines, which makes comparing diagnostic criteria, treatment options, and clinical outcomes difficult. The paucity of data in this patient population extends to treatment of AMR, which remains inconsistent across institutions. Because AMR is a result of the efferent limb of the adaptive immune system being activated secondary to circulating anti-donor antibody, treatment modalities employed are multimodal and focus on removal of anti-donor antibody (Table 3). Treatment mainstays usually include removal and neutralization of antibody through plasmapheresis (PP) and IV immunoglobulin (IVIG) (Levine et al. 2016; Ensor et al. 2017b; Kulkarni et al. 2015; Muller et al. 2018; Neuhaus et al. 2021). IVIG can be administered after and/or between PP sessions. Doses are weight based and range from 100 to 1,000 mg/kg/dose. Additional drugs for immunomodulation targeting further upstream within the adaptive immune system

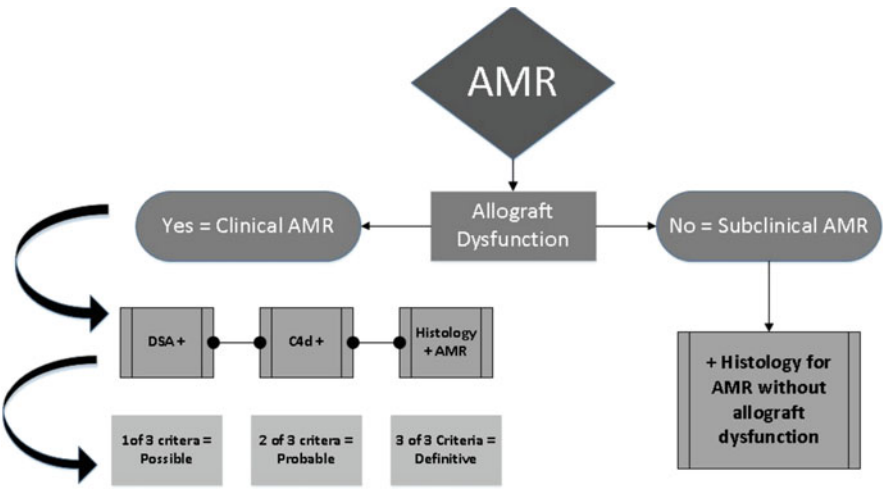

Fig. 1 AMR diagnostic grouping (adopted from Levine et al. 2016)

are often also utilized in combination with IVIG + PP. They include corticosteroids, B-cell depletion with rituximab, targeting plasma cells through proteasome inhibition with bortezomib or carfilzomib, and terminal complement (C5) blockade with eculizumab (Ensor et al. 2017b; Kulkarni et al. 2015; Muller et al. 2018; Neuhaus et al. 2021; Bery and Hachem 2020; Pham et al. 2021). After acute treatment of AMR, additive therapy with ECP has shown promise for chronic management of DSA, and clinical stabilization, but like other therapies it has not been rigorously studied (Benazzo et al. 2020).

Outcomes for AMR treatment vary greatly, and no single treatment regimen has proven to be superior to another. One of the more common regimens for AMR, Rituximab + IVIG + PP, has shown varying antibody responses with patients still often progressing to CLAD and BOS (Otani et al. 2014). Neuhaus et al. employed varying regimens with mixed outcomes. Of 51 patients approximately half received Rituximab + IVIG + PP, other patients received IVIG-based therapy with or without other immunomodulatory medications and PP. Following treatment nearly one-quarter of the patients progressed to allograft failure (Neuhaus et al. 2021).

Ensor et al. evaluated carfilzomib, a non-reversible proteasome inhibitor targeting plasma cells for the treatment of AMR in 14 lung recipients. Primary outcome of this study was to assess differences in those that lost C1q binding affinity after treatment (responders) vs. those that maintained C1q binding affinity (non-responders). Overall, 10 of 14 patients responded to carfilzomib-based therapy. Although the responders to therapy had less progression to BOS, 50% (7/14) of patients ultimately died of BOS, or RAS, greater than 120 days post-carfilzomib therapy (Ensor et al. 2017b).

These data highlight the difficulty of AMR recognition, diagnosis, and treatment post-transplantation. Within this population, the lack of data results in difficultly standardizing treatments and targeted outcomes. Randomized-controlled trials are needed in order to establish gold-standard treatment regimens and improve clinical outcomes with AMR in lung transplantation.

4.3 Chronic Lung Allograft Dysfunction

Despite numerous advancements in lung transplantation and a clear survival benefit in those with end stage lung disease, CLAD is the leading cause of long-term mortality post-transplant. Approximately 50% of lung transplantation recipients will develop CLAD by 5 years post-transplant. Median survival after lung transplantation is 6 years, with CLAD being the major barrier (Valapour et al. 2021; Verleden et al. 2019). The diagnostic criteria of CLAD are substantial ($\geq$20%) and persistent ($>$3 months) decline in FEV_1 from baseline, after alternative diagnoses have been excluded, including ACR, disease recurrence, anastomosis stenosis, and infection (Verleden et al. 2019). CLAD can be further classified into the following phenotypes: BOS, RAS, mixed, or undefined. BOS is the predominant phenotype accounting for approximately 70% of CLAD and is characterized by airflow obstruction, whereas RAS accounts for ~30% and manifests as airflow restriction (Verleden

et al. 2019). Mixed CLAD demonstrates characteristics of both. Depending on the phenotype, responses to therapy may vary.

CLAD management focuses on an attenuation of decline in allograft dysfunction. Mitigating CLAD risk factors and optimization of maintenance immunosuppression is key in preventing subsequent pulmonary decline. CLAD risk factors include ACR; antibody mediated rejection (AMR); gastroesophageal reflux disease (GERD); and some viral, bacterial, and fungal infections. Therefore, post-transplant emphasis should be placed on continuous adherence assessments, frequent FEV_1 monitoring with prompt investigation upon decline, donor specific antibody testing, and employment of appropriate opportunistic/fungal infection prophylaxis and treatment. In patients with documented GERD, fundoplication may be beneficial in delaying pulmonary decline. Escalation of maintenance immunosuppression should be balanced with preventing over-immunosuppression and subsequent infection since both rejection and infection episodes can lead to subsequent decline in pulmonary function (Meyer et al. 2014).

Non-pharmacologic therapies, particularly with BOS, include extracorporeal photopheresis (ECP) and total lymphoid irradiation (TLI). Although data are limited, ECP has a better-established role in the BOS phenotype compared to RAS where an attenuation in the decline of FEV_1 and stabilization of pulmonary function have been observed (Vazirani et al. 2021; Verleden et al. 2009). TLI may reduce allograft decline in those with BOS phenotype who are rapidly deteriorating without response to other therapies. It can also potentially serve as a bridge to re-transplantation (Lebeer et al. 2020).

Pharmacologic treatment options are limited for CLAD management. There are currently no pharmacotherapies with FDA-labeled indications for the treatment of CLAD. Majority of pharmacologic treatment options have been trialed in patients with BOS. Azithromycin, a macrolide antibiotic, possesses immunomodulatory effects that can play a role in lowering neutrophils and cytokines in inflammatory airways diseases. Practice varies on timing of azithromycin initiation which includes either azithromycin prophylaxis (AP) employed shortly after lung transplantation or azithromycin therapy initiated at CLAD diagnosis. ISHLT consensus advises trialing a course of azithromycin for at least 8 weeks following a new diagnosis of CLAD (Verleden et al. 2019). AP has shown improved survival and baseline lung allograft dysfunction, albeit with mixed evidence on reduction in CLAD development rates (without differences in phenotypes observed) (Li et al. 2020; Ruttens et al. 2016; Vos et al. 2011). Evidence of azithromycin therapy in CLAD supports improvement/normalization in FEV_1, with a survival benefit more pronounced with early CLAD-BOS stages (Corris et al. 2015; Gerhardt et al. 2003; Jain et al. 2010; Verleden and Dupont 2004). Additionally, clinical benefit appears to be superior in a subset of patients with high levels of neutrophils found on bronchoalveolar lavage (BAL) (Vos et al. 2011). Patient-specific factors should be investigated before initiating azithromycin therapy, including untreated non-tuberculosis mycobacterium infection, as it would be prudent to avoid macrolide monotherapy to prevent antimicrobial resistance. Other side effects of azithromycin to assess alongside patient-specific factors include prolonged QT, ototoxicity, and liver injury.

Additional CLAD salvage/investigational therapies include montelukast, alemtuzumab, aerosolized cyclosporine, and anti-fibrotic agents. Montelukast, a leukotriene inhibitor, may slow decline, especially in early stages of BOS. However, there is limited evidence on impact of progression and overall survival (Verleden et al. 2011; Vos et al. 2019). Salvage therapy with alemtuzumab has been trialed for refractory BOS. Transient benefit in pulmonary function has been observed (Ensor et al. 2017a). Monoclonal antibody therapy needs to be well balanced with the risks of over-immunosuppression, most notably infection. A reduction in maintenance immunosuppression (specifically, removal of a DNA synthesis inhibitor) and reinitiating antiviral and antifungal prophylaxis post alemtuzumab therapy has been employed in practice. A report of possible CLAD-RAS phenotype revealed resolution of interstitial and alveolar septal fibrosis, however long-term follow-up needs to confirm these findings (Kohno et al. 2011). Antifibrotic agents, such as pirfenidone and nintedanib, are under investigation with small studies/case reports revealing attenuation of decline in lung function in CLAD-RAS. Unfortunately benefit may be limited by gastrointestinal adverse effects (Vos et al. 2013, 2018; Suhling et al. 2016).

In well-selected patients, re-transplantation may be the only treatment option available for the advanced CLAD. Pulmonary and extra-pulmonary risk factors for CLAD should be closely monitored and continuously assessed post-transplant. Since several pharmacologic therapies have demonstrated their greatest impact in early stages of CLAD, it is prudent that a timely diagnosis is made to further drive appropriate management. As CLAD remains the largest barrier to long-term survival post-transplant, concerted efforts should be placed on honing optimal management and development of efficacious pharmacotherapy strategies.

5 Summary

Given advancements in immunosuppression, a multitude of agents, and paucity of high-quality data, treatment is optimized and individualized to each recipient, based on each center's experience and protocols. Unfortunately, even with these advancements medication access is often limited by the lack of FDA-approval and compendia recognition for use of these agents in lung transplantation. Assessing outcomes for these agents are further complicated by heterogeneity of data, as well as short allograft lifespan. Also, definitions for rejection and CLAD have changed over time creating a challenge when comparing literature. Given that large randomized-controlled trials of immunosuppressive drugs in lung transplantation are lacking, much of the practices are extrapolated from abdominal transplantation and are often institution specific. Because of this, institutional collaboration and networking is of high importance and will continue to drive optimal immunosuppression regimens until higher quality data are available.

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Immunosuppression and Kidney Transplantation

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Contents

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Abstract

Immunosuppression is complex, fraught with on-target and off-target adverse effects, and hard to get right but is the key to successful allotransplantation. Herein, we review the key immunosuppressive agent classes used for kidney transplant, highlighting mechanisms of action and typical clinical use.

Keywords

Antibody mediation rejection · Calcineurin inhibitors · Immunosuppression · Induction therapy · Kidney transplantation · Rejection · T-cell depleting agents · Transplant protocols

1 Introduction

The first successful kidney transplant was pioneered in 1954 by Joseph Murray at Harvard, a living donor transplantation between identical twins. Subsequently, it was the development and initiation of immunosuppressive medications that made organ transplantation between genetically dissimilar individuals possible. Multiple therapeutic options have emerged since, because of our better understanding of the immune response mechanisms that led to lower rejection rates, and better graft and patient survival in kidney transplantation.

1.1 Brief History

Among the first immunosuppressive strategies used was total body radiation. Along the same period, the anti-inflammatory properties of cortisone in patients with rheumatoid arthritis were discovered (Hench et al. 1949). Thereafter, prednisone was routinely combined with azathioprine which was introduced in early 1960s (Calne et al. 1962; Murray et al. 1963; Zukoski et al. 1960). In the 1970s, anti-thymocyte (ATG) globulin and antilymphocyte globulin (ALG), polyclonal antibody preparations were introduced and a typical kidney transplant immunosuppression protocol consisted of an induction regimen with ALG with prednisone and azathioprine being used for maintenance immunosuppression (Fig. 1).

Cyclosporine A, an extracted compound of the fungus *Tolypocladium inflatum (*Köhler and Milstein 1975*)*, was discovered in the early 1980s. This groundbreaking discovery revolutionized kidney transplant outcomes with a 30 to 40% reduction in rejection rates, and >80% graft survival at 1 year (Zand 2005). This dramatic benefit was easy to recognize considering the poor outcomes prior to its introduction. Cyclosporine was coupled with prednisone and oftentimes azathioprine was being added to constitute the "triple therapy." Major advancements to follow were the introduction of tacrolimus into liver transplantation and later to kidney transplant (Pirsch et al. 1997) as an alternative to cyclosporine and mycophenolate mofetil

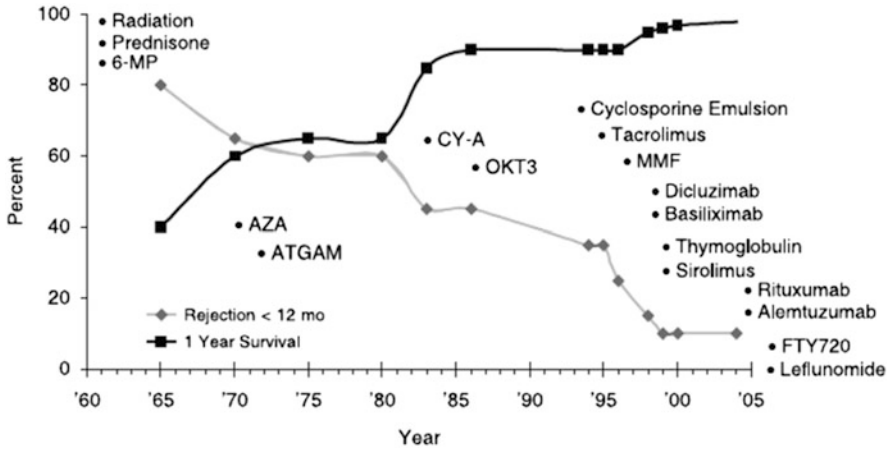

Fig. 1 The development of more potent immunosuppression medications over the years leads to lower rejection rates and subsequently better graft survival (Zand 2005)

which was superior to azathioprine with lower rejection episodes when used with cyclosporine and prednisone (Knight et al. 2009). Another key development was the introduction of the first monoclonal antibody (mAb) to be used in clinical medicine, OKT3 in 1985. It was being used for steroid-resistant rejections and occasionally as an induction agent. Of similar use, basiliximab and daclizumab two humanized monoclonal Il-2 inhibitors receptor antagonists (IL2-RA) were later introduced.

Sirolimus was introduced in 1999 as mammalian target of rapamycin (mTOR) pathway inhibitor, a new class of medications with antineoplastic properties (Shimobayashi and Hall 2014) in addition to immunosuppressive potential. The last major medication that gained FDA approval in 2011 was belatacept, which works through competitive costimulation blockade (Larsen et al. 2005).

1.2 Current Practices

With the multiplicity of immunosuppressive agents and the rapid advances in kidney transplant immunosuppression, a wide variety of treatment protocols and clinical practices are adopted at different transplant centers throughout the USA.

According to the organ procurement and transplantation network, scientific registry of transplant recipients data report, T-cell depleting agents remain the most common induction agent in 2018 used in 75% of cases. Twenty percent of kidney transplant used IL2-RA as induction agent and the remaining 5% didn't use any (Hart et al. 2020).

In regard to maintenance immunosuppression, tacrolimus and mycophenolate mofetil based regimen constitutes the major regimen used. Approximately 30% are steroid free regimens, a stable proportion over the past years. Ten percent of cases are non-calcineurin based regimens, mainly belatacept based. (Hart et al. 2020).

1.3 Alloimmune Reaction Targets

The understanding of the different mechanisms of the immune response, including B- and T-cell development, activation and proliferation, cytokine signaling, and complement activation contributed to the advancement of new therapeutics and vice versa. The target of the immunosuppressive agents can be divided based on the stage of the immune reaction. "Signal 1" is activated when an antigen (recipient HLA peptides) on the surface of antigen presenting cell (APC) (most commonly dendritic cell) triggers T cells via the CD3 complex. Costimulation or "signal 2" constitutes the interaction of CD80 and CD 86 (B7) on the surface of APC and CD28 on T cells. Both signal 1 and 2 are necessary to activate three signal transduction pathways: the calcium-calcineurin pathway, the RAS-mitogen activated protein (MAP) kinase pathway, and the nuclear factor-kb pathway (Wang et al. 2004). Transcription factors that trigger IL2, CD 25 (IL2 a subunit), and CD145 expression are then activated. IL 2 subsequently activates the target of mTOR pathway which constitutes "signal 3," the trigger for cell proliferation. Nucleotide synthesis, another target for immunosuppressive medications, is also required for lymphocyte proliferation and the mobilization of effector T cells. B cells are engaged when an alloantigen interacts with their antigen receptor in secondary lymphoid tissues, the lymphoid follicles or the red pulp of spleen, for example (MacLennan et al. 2003), or the kidney allograft itself (Sarwal et al. 2003) producing antibodies against the HLA antigens. The main agents of kidney allograft rejection are effector T cells and anti-HLA alloantibodies. In general, immunosuppression can be achieved by depleting lymphocytes, blocking their response pathways, slowing down the production and neutralizing the effect of alloantibodies.

This chapter will be divided into three sections according to the clinical use of each immunosuppressive medication. Immunosuppressive agents used for induction and maintenance immunosuppression, and rejection treatment (mainly antibody mediated rejection) with focus on those that are currently used in kidney transplantation will be reviewed.

2 Induction Therapy

Induction regimens are part of the immunosuppression protocols in over 80% of kidney transplant centers in the USA. The use of induction agents reduces the rate of acute rejection and subsequently improves short-term graft survival, however, there is no prospective data clearly demonstrating a superior outcome in long-term graft survival. Induction therapy seems to be clinically indicated in early steroid withdrawal protocols where maintenance immunosuppression is being minimized. Induction therapy is warranted in high immunologic risk individuals (high calculated panel of reactive antibody (cPRA), positive cross match transplants, positive donor specific anti-HLA antibodies (DSA), prior transplant recipients, recipient of black race) and those whom a delayed graft function is expected because of donor characteristics or high cold ischemia time.

Induction agents are divided into T-cell depleting agents – monoclonal and polyclonal anti-thymocyte globulins (ATG) and alemtuzumab and non-T-cell depleting – interleukin 2 receptor antagonist (IL2RA). In addition to their use as induction agents, T-cell depleting agents are used to treat T-cell mediated rejection.

IL2RA use is limited to kidney transplant recipients with low immunologic risk as ATG has been shown to be more effective in preventing acute rejection in the high-risk group (Brennan et al. 2006). Whereas, alemtuzumab had similar outcomes compared to ATG and was superior to IL2RA (Hanaway et al. 2011).

2.1 T-Cell Depleting Agents

2.1.1 Monoclonal Antibodies

Monoclonal antibody muromonab-CD3 (OKT3): OKT3 is the first mAbs approved by the FDA for use in humans in 1986 for prevention of rejection in kidney, heart, and liver transplant (OMTS Group 1985). It is an anti-T-cell receptor (TCR) antagonist that targets the CD3 subunit of the TCR complex inhibiting the first point of antigen presentation (targeting signal 1). It is a murine antibody, thus results in significant side effects related to its mitogenicity which are potentially fatal first-dose reactions. In efforts to minimize its mitogenicity, humanized forms of anti-TCR mAbs that target other subunits (Larsen et al. 2005; Hart et al. 2020; Wang et al. 2004) have been developed but their production has been on hold given ongoing safety and efficacy concerns.

2.1.2 Polyclonal Anti-thymocyte Globulin

Therapeutic antilymphocyte polyclonal antibodies are produced by immunizing with human thymocytes either horses (eATG (equine), ATGAM) or rabbits (Thymoglobulin-Genzyme), or immunizing rabbits with lymphocytes from a Jurkat cell leukemia line (Fresenius antithymocyte globulin [ATG]). Two forms of rabbit anti-thymocyte globulin (rATG) are available depending on the cell type used for rabbit immunization, thymoglobulin (Genzyme) which is available in the USA and anti-T-lymphocyte immune globulin (ATG-Fresenius) used in Europe. In small head-to-head trials, thymoglobulin was superior to ATG-Fresenius in regard to both efficacy and side effects (Gharekhani et al. 2013). rATG is the primarily used antilymphocyte in clinical practice whereas ATGAM, although available, is not widely used partly because it is less potent.

Rabbit Anti-Thymocyte Globulin

Specialized rabbits are immunized with thymocytes or activated human T cells and the resultant IgG fraction of the sera is purified to remove irrelevant antibody materials. These antibodies are polyclonal as directed against multiple thymocyte antigens. Its mode of action is not fully characterized, but rATG antibodies are predominantly anti-T lymphocytes and will cause T-cell depletion via complement-dependent cytotoxicity and T-cell activation-induced apoptosis (Zand et al. 2005) or can be cleared by the reticuloendothelial system. Since some antigens are shared

among T cells and other immune cells, rATG exhibits some activity against B cells, monocytes, and to a lesser neutrophils. Most importantly, rATG causes a sustained expansion of regulatory T cells which maintain immune balance and prevent acute rejection.

Dose and Administration: rATG is administered at 1.5 mg/kg doses with a cumulative dose ranging between 3–6 mg/kg depending on recipient characteristics and center practice. It is more effective when used in the operating room prior to anastomosis of the graft. Allergic reactions are prevented by administering premedication consisting of steroids and diphenhydramine. It is administered through a central vein over 4–8 h. When using a peripheral vein, it might be associated with vein thrombosis or thrombophlebitis which can be prevented by adding heparin and hydrocortisone to the infusion.

Adverse Reactions: The side effects associated with rATG administration are chills, fever, and arthralgia, commonly seen with polyclonal antibody preparations. Serum sickness is seen but rarely, because the continued immunosuppression reduces immune complex formation and deposition. Cytokine release syndrome (with pulmonary edema and hypotension) is the most worrisome. Anaphylaxis can be seen, especially with patients with prior history of rabbit sensitivity.

Leukopenia, a direct consequence of T-cell depleting therapy, and thrombocytopenia are seen. The subsequent dose is usually halved or held with a platelet count of 50,000 to 75,000 cells/mL or a white blood cell count of 2,000 to 3,000 cells/mL.

Cytomegalovirus (CMV) infection is a late manifestation of rATG use. This is usually prevented by the use of CMV prophylaxis with valganciclovir for 3–-9 months (depending on donor and recipient serostatus and rATG dose) after administration especially in high-risk populations (donor with CMV positive serostatus and recipients with negative serostatus). Post-transplant lymphoproliferative disorder, particularly EBV related lymphoma is an infrequent but grave consequence.

2.1.3 Alemtuzumab

Alemtuzumab (Campath 1H) is a humanized mAb, DNA-derived directed against CD52, a cell surface glycoprotein of unclear physiologic significance, present on both B- and T-cell lymphoid cell line. It was initially approved for the treatment of refractory chronic lymphocytic leukemia (Alinari et al. 2007) and reintroduced in 2012 as a treatment for multiple sclerosis (Freedman et al. 2013). The use of Alemtuzumab in kidney transplantation as an induction agent is “off-label.” It is administered as a single dose of 30 mg intraoperatively and has fewer infusion-related reactions as a humanized antibody. Its ease of administration and fewer side effects coupled with a comparable efficacy make it an attractive alternative to ATG. Alemtuzumab induces a significant, durable T-cell depletion up to 6–12 months after administration. The infectious and malignancy risks are similar to other T-cell depleting agents.

2.2 Interleukin 2 Receptor Antagonists

Once T lymphocytes become activated in response to signal 1 and signal 2, they express CD25, the α-subunit of the IL2 receptor. Subsequently, IL-2 will lead to the intracellular signaling and proliferation of T cells. Basiliximab (Simulect) and daclizumab (Zenapax) are anti-CD25 monoclonal antibodies targeted against the α-subunit that will prevent T-cell proliferation. Daclizumab is no longer in production for clinical kidney transplantation. Basiliximab reduces the risk of acute rejection in patients with lower immunologic risk. Although it originates as a murine monoclonal antibody, 75% of it has been replaced by human IgG, thus it is well tolerated and does not induce a first-dose reaction. Basiliximab half-life is prolonged (longer than 7 days) as it doesn't induce antimurine antibodies and is given as an intravenous dose of 20 mg twice. The first intraoperatively and the second 4 days after. IL2 R sites are usually saturated for 30–45 days.

3 Maintenance Therapy

Long-term immunosuppression regimens have changed significantly over the last decades and the number of agents available significantly increased. The aim of maintenance immunosuppression goes beyond the prevention of acute rejection, to the minimization of total immunosuppression and management of chronic allograft rejection and nephropathy. The results of the symphony trial where three major agents were compared cyclosporine, tacrolimus and sirolimus still govern our clinical practice to this day (Ekberg et al. 2007). Tacrolimus was shown to be superior to cyclosporine and sirolimus. Thus, it is the first-line agent in most transplant center protocols. It is generally coupled with mycophenolate which has substituted azathioprine given its superior outcome (Knight et al. 2009). Belatacept, a costimulatory blockade agent, is an alternative for calcineurin-inhibitor based regimens with promising outcomes (Vincenti et al. 2016).

3.1 Calcineurin Inhibitors

Calcineurin inhibitors (CNIs) remain the cornerstone of immunosuppression regimen used in most transplant centers for the past 30 years. The two main calcineurin inhibitors used are cyclosporine and tacrolimus. An investigational drug, voclosporin has been recently studied in lupus nephritis (Arriens et al. 2020). Cyclosporine and tacrolimus are similar not only in regard to their mechanism of action, but also in their clinical efficacy and adverse event profile. Nonetheless, they are biochemically distinct and have discrete differences.

They are both isolated from fungus species. Cyclosporine is an 11-amino acid cyclic polypeptide extracted from *Tolypocladium inflatum* (Köhler and Milstein 1975). Tacrolimus is a macrolide antibiotic compound isolated from *Streptomyces*

tsukubaensis. Its name is still oftentimes substituted by its laboratory designation FK506.

3.1.1 Mechanism of Action

CNIs inhibit the immune response by targeting signal 1. A calcineurin-dependent pathway is triggered after the initial binding of the APC to the TCR, that is necessary for initial gene transcription and subsequently additional T-cell activation. When CNIs are administered, cyclophilin in cyclosporine and tacrolimus-binding protein (FKBP) in tacrolimus bind to their cytoplasmic receptor proteins which in turn bind to calcineurin and inhibit its function. Calcineurin is a phosphatase which dephosphorylates nuclear regulatory proteins, particularly nuclear factor of activated T cells in the setting of immune response, facilitating their entry to the nucleus. CNIs thus inhibit calcineurin-dependent gene transcription including several critical cytokine genes (IL-2, IL-4, Interferon-γ, and tumor necrosis factor-α) and downstream lymphocyte proliferation.

They are unique when compared to their predecessors as they selectively inhibit the immune response. At a therapeutic level, the calcineurin activity is reduced by 50%; this allows for a degree of immune responsiveness to maintain appropriate host defense.

3.1.2 Dose and Administration

Cyclosporine: The original, non-modified form, oil-based Sandimmune has a great variability in absorption and has been substituted by the microemulsion, Neoral/Gengraf. Both are available in 25 mg and 100 mg capsules that are administered twice daily. Intravenous form is administered twice daily in a 4-h infusion. The conversion from po form is 3:1.

Initial dose is 9 mg/kg/day adjusted according to the target level which varies according to the different stages of transplant. A peak-level 2 h after dosing is the most accurate and consistent. It correlates better with drug exposure than a 12 h trough level, although the latter is more often used for convenience.

Tacrolimus: The immediate release (IR) preparation Prograf is available in 0.5 mg, 1 mg, and 5 mg capsules typically administered twice a day. IV formulations are available and the conversion is equal to one-third to one-fourth of the oral dose. It is less commonly used, as tacrolimus can be given sublingually when a po route is unavailable. Newer long-acting preparations are available – ER-tacrolimus (Astagraf) in 0.5, 1, and 5 mg capsules and LCP-tacrolimus (Envarsus) in 0.75, 1, and 4 mg tablets. These once-daily formulations improve medication compliance. LCP-tacrolimus requires 30% reduction from prograf dose as it has better bioavailability along with a decreased peak level (Budde et al. 2014; Tremblay et al. 2017). IR-tacrolimus is typically started at 0.05–0.1 mg/kg/day adjusted by 12 h trough level.

3.1.3 Metabolism and Drug–Drug Interaction

Cyclosporine and tacrolimus are both metabolized via cytochrome P450 (CYP) 3A4 and 3A5 in the liver, small intestine, and in the kidney to lesser extent.

P-glycoprotein (P-gp), an efflux pump that transports substances across the intracellular and extracellular membranes is also involved in CNIs metabolism. P-gp is found in hepatocytes, distal and proximal renal tubular cells, intestinal epithelium, and the luminal surface of capillary endothelial cells in the brain. In the gut, P-gp reduces the bioavailability of CNIs as they are repeatedly taken up and transported out of enterocytes. Polymorphisms in P-gp and CYP3A5 cause significant interpersonal drug level variability by affecting drug absorption, metabolism and distribution. This variability potentially influences drug efficacy and toxicity as it will affect its concentration at target sites. CYP3A5*1 allele (Kuehl et al. 2001) found predominantly in individuals of African descent encodes for a CYP3A5 enzyme that is associated with rapid metabolism of CNIs and subsequently lead to increased dose requirements as opposed to individuals who carry CYP3A5 *3/*3 alleles (Barbarino et al. 2013) that encode for a non-functional CYP3A5 protein and thus have reduced dose requirements.

Any drug that impacts CYPA3A4/5 or P-gp activity has a potential interaction with CNIs. Inducers of CYP3A activity will decrease CNIs concentration. These are anti-tuberculous drugs – rifampin and rifabutin; anticonvulsants – barbiturates, phenytoin, and carbamazepine; antibiotics – nafcillin; herbal preparation – St. John's wort; corticosteroids – tacrolimus level may increase by 25% after steroid discontinuation. CYP3A inhibitors increase CNI concentration. Drugs that raise CNIs levels are – non-dihydropyridine calcium channel blockers – diltiazem and verapamil; antifungals, all azole derivatives – ketoconazole, fluconazole, itraconazole, voriconazole, and isavuconazole; macrolide antibiotics – erythromycin and clarithromycin; antiretroviral therapy, mainly protease inhibitors – ritonavir; food – grapefruit juice. CYP3A inhibitors are occasionally added to boost CNI levels when a therapeutic level is not achieved despite using high CNI doses. Aside from medications, diarrhea and bowel inflammation significantly increase CNI levels due to decreased P-gp and CYP3A4 function in enterocytes.

3.1.4 Adverse Events

Kidney Related – Calcineurin Inhibitor Nephrotoxicity: CNI use may lead to significant nephrotoxicity. Acute CNI toxicity occurs early after kidney transplant and is often reversible with dose reduction (Thölking et al. 2017). There are three major acute nephrotoxicity manifestations: vascular vasoconstriction, tubulopathy, and thrombotic microangiopathy (TMA). CNIs cause endothelial cell injury and afferent arteriole vasoconstriction mediated by the production of vasoconstrictors such as endothelin, activation of renin-angiotensin II system, and inhibition of vasodilators such as nitric oxide and cyclooxygenase-2 (Naesens et al. 2009). This vascular effect is reversible and manifest as hypertension and decreased glomerular filtration rate. CNIs may lead to acute tubular damage, whose mechanism is not completely understood but could be related to direct toxicity affecting the endoplasmic reticulum and mitochondria (Pallet et al. 2008). A rare but more severe complication is thrombotic microangiopathy attributed to endothelial injury, causing platelet aggregation and activation of the coagulation cascade (Ponticelli 2007). Electrolytes disturbances are commonly encountered, similar to what is seen in Gordon

syndrome- pseudohypoaldosteronism with hypertension, metabolic acidosis and hyperkalemia even with normal kidney function. Chronic CNI nephrotoxicity occurs several months post-transplant due to cumulative and persistent vascular damage. Clinically, it manifests as hypertension, worsening kidney function and proteinuria and histologically by hyaline arteriolopathy, stripped tubulointerstitial scarring, and glomerulosclerosis (Nankivell et al. 2016).

Non-renal: Some manifestations differ among tacrolimus and cyclosporine particularly cosmetic complications. Cyclosporine is associated with hypertrichosis, and gingival hyperplasia whereas tacrolimus causes hair loss and alopecia. Metabolic complications include hyperlipidemia, more often seen with cyclosporine and post-transplant glucose intolerance and new-onset diabetes more so with tacrolimus which is toxic to the pancreatic islet cells. Neurotoxicity ranging from tremor, dysesthesias, headache is common and is level related.

3.2 Antimetabolites

3.2.1 Mycophenolic Acid

Mycophenolic acid (MPA) is a fermentation product of several *Penicillium* species. It is the active compound of the prodrug mycophenolate mofetil (MMF) (CellCept) that was introduced to kidney transplantation in 1995. It is available in 250 mg capsules and 500 mg tablets and the typical dose is 1 g twice daily. Myfortic is an enteric-coated form of MPA that became available in 2004 in two formulations 180 mg (equivalent to 250 mg of MMF) and 360 mg tablets. MPA is an inhibitor of the enzyme inosine monophosphate dehydrogenase (IMPDH), the rate limiting enzyme critical for de novo purine synthesis and thus DNA synthesis in T and B cells. Lymphocytes rely on de novo DNA synthesis more than other cell types that have a salvage pathway for guanosine nucleotide synthesis from guanine. It has been demonstrated that MPA blocks the proliferation of T and B lymphocytes and subsequently inhibits antibody formation and generation of cytotoxic T cells (Danovitch 2005). Primary side effects are gastrointestinal and hematopoietic. Diarrhea occurs in one-third of patients along with nausea, dyspepsia, and vomiting in up to 20% of patients. GI side effects are more frequently encountered with dosage >1 g twice daily. Hematopoietic side effects include leukopenia, anemia, and thrombocytopenia despite being specific to lymphocytes. These are seen at a similar rate to azathioprine. MPA is teratogenic and should be discontinued six weeks prior to planned pregnancy and substituted to azathioprine.

3.2.2 Azathioprine

Azathioprine (AZA) (Imuran) is an antimetabolite, an analog of the early immunosuppressant, 6-mercaptopurine. This metabolite acts as a purine analog that interferes with de novo purine and subsequently, DNA and RNA synthesis inhibiting gene replication and T-cell activation (Elion 1989). Its regular dose when used in conjunction with a CNI is 1–2 mg/kg. AZA is a bone marrow suppressant thus its hematologic side effects (anemia, thrombocytopenia, and leukopenia). Its

concomitant use with xanthine oxidase inhibitors (allopurinol and febuxostat) slows 6-mercaptopurine elimination and exacerbates these side effects (Berns et al. 1972). AZA is safe with pregnancy unlike MPA (Sifontis et al. 2006).

3.3 mTOR Inhibitors: Everolimus and Sirolimus

Clinically available mTOR inhibitors are sirolimus and everolimus. Sirolimus (Rapamune) is a macrolide antibiotic produced by *Streptomyces hygroscopicus* and is structurally related to tacrolimus, available in 0.5, 1, or 5 mg tablet. Everolimus (Zortress) is a derivative of sirolimus with different pharmacokinetics, available in 0.25, 0.5, and 0.75 mg tablets. The mammalian target of rapamycin (mTOR) pathway constitutes signal 3 of the immune response and will lead to cell cycle progression from G1 to S and proliferation in response to cytokine stimulation (mainly IL-2). mTOR inhibitors bind to FKBP (the same cytoplasm-binding protein that binds tacrolimus) and the complex engages with mTOR, a regulatory kinase, and inhibits its actions causing reduced cytokine-dependent cellular proliferation. mTOR signaling is ubiquitous, and not exclusive to lymphocytes and has been described in monocytes, dendritic cells, natural killer cells, as well as nonhematopoietic cells (endothelial cells, fibroblasts, hepatocytes, and smooth muscle cells) (Ferrer et al. 2011). In addition to its immunosuppressive effects, the inhibition of mTOR will lead to anti-proliferative, antiviral, anti-inflammatory, and antitumor effects (Peddi et al. 2013).

Similar to CNIs, mTOR inhibitors have nephrotoxic side effects. In addition to mTOR kinase, mTOR inhibitors also target the vascular endothelial growth factor (VEGF) (Guba et al. 2002; Knoll et al. 2014) inhibiting its activity, causing podocyte damage and eventually proteinuria and nephrotic syndrome (Diekmann et al. 2012). Other nephrotoxic effects include focal segmental glomerulosclerosis, TMA, acute tubular injury, and atypical casts (when combined with tacrolimus) (Smith et al. 2003). mTOR inhibitors prevent wound healing so should be avoided in fresh transplant recipients and be switched to CNIs 6 weeks prior to major surgery or immediately postoperatively for emergent surgery. Other side effects include edema, hypertension, gastrointestinal side effects – mouth ulcers, diarrhea, hyperlipidemia (hypercholesterolemia and hypertriglyceridemia), hyperglycemia, and cytopenia (mainly thrombocytopenia and anemia).

3.4 Corticosteroids

Corticosteroids, one of the first immunosuppression medications used, still play a central role in kidney transplantation. Steroid avoidance or withdrawal protocols have been developed, and when steroids are used, their dose is small, typically equivalent to prednisone 5 mg daily. Steroid receptor is expressed on most mammalian cells and modulates a multitude of cellular functions. Corticosteroids diffuse intracellularly and bind to their cytoplasmic receptor, the complex translocates to the

nucleus where it binds to DNA sequences – glucocorticoid response element (GRE), responsible for cytokine gene transcription, and blocking its action. It also inhibits other cytokine transcription factors such as nuclear factor-Kb (Rhen and Cidlowski 2005). As a result, the expression of IL-1, IL-2, IL-3, IL-6, TNF-a, and IFN-g is inhibited with the downstream result of T-cell depletion, inhibition of Th1 differentiation, induction of apoptosis, and macrophage dysfunction.

3.5 Belatacept

Belatacept (Nulojix) is a costimulatory blockade agent targeting signal 2 of the immune response. After TCR binding, optimal T-cell activation requires a costimulation signal conferred by the interaction of CD80/86 on APC and CD28 on T cell. After an effective T-cell response, cytotoxic T lymphocyte-associated protein 4 (CTLA4) competitively binds CD80/86 and downregulates the cell activity. Belatacept is a human fusion protein containing CTLA4 linked to Fc domain of human IgG1. Belatacept was demonstrated to be noninferior to cyclosporine in terms of patient and graft survival and has the potential to replace CNI-based immunosuppressive protocols. Belatacept is available as an intravenous formulation. When administered de novo at time of transplant, it is given at a dose of 10 mg/ kg on day 1, 5, 15, 28, 56 and then at 5 mg/kg q 28 days (Adams et al. 2017). Despite a higher risk of rejection, patients on belatacept have higher GFR, graft, and allograft outcomes and appear to develop fewer de novo DSA antibodies (Vincenti et al. 2016). It is well tolerated with few metabolic complications. EBV naïve patients are at risk for post-transplant lymphoproliferative disorder, thus its use is restricted to patients with positive EBV serology.

4 Antibody Mediated Rejection

Antibody mediated rejection (AMR) is a severe form of rejection resistant to standard treatment with immunosuppressant medications. Post-transplant AMR, chronic active AMR (CAAMR), and transplant glomerulopathy (TG) remain a significant problem in kidney transplantation leading to long-term graft failure. Will briefly discuss the therapeutic approaches used for the treatment of AMR.

1. *Intravenous Ig (IVIG)*: an IgG rich Ig extract pooled from thousand donors. IVIG immunosuppressive mechanisms are broad, including the direct binding to antibodies, superantigens and pathogens, inhibition of complement fixation, and stimulation of FcR-induced anti-inflammatory pathways.
2. *Rituximab*: a chimeric mAb against CD20 that is expressed on pre-B and mature B cells but not differentiated plasma cells leading to B-cell depletion via complement-dependent cytotoxicity, growth arrest, and apoptosis (Pescovitz 2006). Humanized (Ocrelizumab) and fully humanized (ofatumumab) anti-CD20 mAbs are available for clinical use.

3. *Anti-Plasma Cell Therapies*: Daratumumab is an anti-CD38 mAb, as CD38 is expressed on plasma cells. Bortezomib and Carfilzomib are proteasome inhibitors.
4. *Tocilizumab*: mAb directed at IL-6 receptor that induces a significant reduction of B-cell hyperreactivity with promising results in CAAMR.
5. *Eculizumab*: C5 inhibitor that prevents cleavage of C5 to C5a and C5ba and the formation of the membrane attack complex C5b-9.
6. *Newer Agents*: a number of agents that target different aspects of the B-cell and complement-mediated aspects of the immune response are coming online soon that have generated considerable excitement within the transplant community. It is felt that combinations of agents may prove more effective at managing acute and chronic antibody mediated alloimmune responses than currently available agents, which remain disappointing. IdeS-IgG-degrading enzyme derived from *Streptococcus pyogenes* (Imlifidase), an endopeptidase, cleaves human IgG into F $(ab')_2$ and Fc fragments inhibiting complement-dependent cytotoxicity and antibody-dependent cellular cytotoxicity permitted transplant between HLA-incompatible individuals by cleaving donor specific antibodies (DSA). Clazakizumab is an immunoglobulin G1 (IgG1) mAb aimed at the IL-6 ligand which is being currently studied for the use in CAAMR along with evidence of TG on kidney biopsy. Anti-C1s (BIVV009) is a novel investigational drug being examined to be used in the setting of C4d+ and C1q+ DSA. Similarly, C1 esterase inhibitor has been shown to prevent TG when used as an adjunct to AMR therapy when compared to placebo (Montgomery et al. 2016).

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Immunosuppression in Rheumatologic and Auto-immune Disease

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Contents

Abstract

Many rheumatologic diseases are thought to originate in dysregulation of the immune system; lupus nephritis, for example, involves humoral immunity, while autoinflammatory diseases such as familial Mediterranean fever are caused by

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defects in innate immunity. Of note, this dysregulation may involve both upregulation of immune system components and aspects of immunodeficiency. Treatment of rheumatologic diseases thus requires a familiarity with a variety of immunosuppressive medications and their effects on immune system function.

In many rheumatologic conditions, due to an incompletely elucidated mechanism of disease, immunosuppression is relatively broad in contrast to agents used, for example, in treatment of transplant rejection. Multiple immunosuppressive drugs may also be used in succession or in combination. As such, an understanding of the mechanisms and targets of immunosuppressive drugs is essential to appreciating their utility and potential adverse effects. Because of the overlap between therapies used in rheumatologic as well as other inflammatory disorders, some of these medications are discussed in other disease processes (e.g., Immunosuppression for inflammatory bowel disease) or in greater detail in other chapters of this textbook (corticosteroids, mTOR inhibitors, antiproliferative agents).

Keywords

Autoimmune · Autoinflammatory · Biologics · DMARDs · Immunosuppressants · Lupus · Rheumatoid arthritis · Rheumatology · Vasculitis

1 Introduction

Many rheumatologic diseases are thought to originate in dysregulation of the immune system; lupus nephritis, for example, involves humoral immunity, while autoinflammatory diseases such as familial Mediterranean fever are caused by defects in innate immunity. Of note, this dysregulation may involve both upregulation of immune system components and aspects of immunodeficiency. Treatment of rheumatologic diseases thus requires a familiarity with a variety of immunosuppressive medications and their effects on immune system function.

In many rheumatologic conditions, due to an incompletely elucidated mechanism of disease, immunosuppression is relatively broad in contrast to agents used, for example, in treatment of transplant rejection. Multiple immunosuppressive drugs may also be used in succession or in combination. As such, an understanding of the mechanisms and targets of immunosuppressive drugs is essential to appreciating their utility and potential adverse effects. Because of the overlap between therapies used in rheumatologic as well as other inflammatory disorders, some of these medications are discussed in other disease processes (e.g., Immunosuppression for inflammatory bowel disease) or in greater detail in other chapters of this textbook (corticosteroids, mTOR inhibitors, antiproliferative agents).

2 Rheumatologic Diseases

The rheumatologic diseases covered in this chapter are not a comprehensive list, but are illustrative of the variety of immunosuppressive agents commonly used as well as the current understanding of disease pathophysiology. As such, some rheumatologic conditions in which unique immunomodulatory therapy is not a mainstay of treatment such as systemic sclerosis and Sjogren's syndrome) are not touched upon, and rheumatoid arthritis, in which many immunosuppressive treatments can be potentially used, receives the most attention. Conditions such as small and large vessel vasculitis, inflammatory myositis, and Sjogren's syndrome, in which immunosuppressive treatments overlap with other disorders, are not separately addressed here.

2.1 Rheumatoid Arthritis

Rheumatoid arthritis (Karie et al. 2008) is a chronic inflammatory arthritis characterized by synovitis, formation of a proliferative synovial pannus, and cartilage and bone degradation mediated by activated fibroblast-like synoviocytes (FLS) and osteoclasts, respectively (Bartok and Firestein 2010). The resulting joint inflammation leads to pain and disability. RA is commonly associated with positive rheumatoid factor and anti-citrullinated peptide antibodies and is thought to be caused by tumor necrosis factor-alpha (TNF-α)-driven activation of endothelial cells and osteoclasts and production of other inflammatory cytokines such as interleukin (IL)-1 and IL-6 (Feldmann and Maini 2003; Thompson et al. 2016).

Currently, immunosuppression is the mainstay of RA treatment, with patients taking conventional disease-modifying anti-rheumatic drugs (DMARDs), biologic DMARDs (monoclonal antibodies and receptor constructs), targeted synthetic DMARDs (tsDMARDs such as Janus-kinase, or JAK, inhibitors), or a combination of these drugs.

Conventional synthetic DMARDs (csDMARDs) used in RA include immunosuppressive medication such as methotrexate (MTX), leflunomide, and azathioprine as well as non-immunosuppressive treatments such as sulfasalazine and hydroxychloroquine (HCQ). Older treatments including penicillamine and gold salts are no longer commonly used due to side effects and availability of more effective therapy.

2.1.1 Conventional Synthetic DMARDs

Though several oral DMARDs are commonly referred to as "antiproliferative," some of these agents may be effective in RA for reasons other than their effect on inflammatory cell proliferation. Low-dose oral MTX (in contrast to high doses used as chemotherapy) is used as first-line therapy in rheumatoid arthritis. Although it is well known as a folate antagonist, inhibiting purine and pyrimidine synthesis and thus cell proliferation (Jolivet et al. 1983), it also has anti-inflammatory effects that may be more essential to its efficacy in rheumatoid arthritis (Cronstein and

Sitkovsky 2017), as exogenous folic acid supplementation does not decrease methotrexate's efficacy in RA (Friedman and Cronstein 2019). Specifically, methotrexate increases release of the anti-inflammatory mediator, adenosine, which may in turn lead to reduction of pro-inflammatory cytokines, inhibition of osteoclast formation, and transition of M1 to M2 macrophages (Cronstein and Sitkovsky 2017).

Methotrexate has several important toxicities that require monitoring, especially in the setting of other conditions or medications that can affect these systems, including pulmonary disease ("methotrexate lung") and liver disease. While it does not cause direct renal toxicity, it is primarily excreted via the kidneys; impairment of renal function beyond chronic kidney disease stage 3 requires dose adjustment (Karie et al. 2008). Common adverse effects of methotrexate therapy include nausea, hair loss, and stomatitis. Routine testing of liver enzymes (for transaminitis) and blood counts (for cytopenias) is essential in long-term use of low-dose oral methotrexate; abnormalities in these tests may require temporary cessation of the medication and/or dose adjustment.

Leflunomide is another commonly used DMARD in RA. Its active metabolite, A77 1726, inhibits the enzyme dihydroorotate dehydrogenase, which is necessary for the rate-limiting step in lymphocyte pyrimidine synthesis; thus, it is thought to lymphocyte proliferation (Breedveld and Dayer 2000) and downstream effects thereof. As with methotrexate, use of leflunomide requires regular monitoring of blood counts and liver enzymes.

Sulfasalazine and azathioprine have more limited efficacy in RA treatment. Unlike methotrexate and leflunomide, however, they are considered relatively safe in pregnancy. Studies of sulfasalazine in RA are limited, but have shown improvement in patients' RA symptoms and markers of inflammation (McConkey et al. 1978; Pullar et al. 1983). Sulfasalazine is metabolized to sulfapyridine and 5-aminosalicylic acid in the large intestine; both sulfasalazine and its metabolites may have immunomodulatory properties in RA, though 5-ASA remains in the large intestine (Das and Dubin 1976). Its exact mechanism of action is as yet unknown, but proposed mechanisms include inhibition of leukotriene production, reduction of free radical generation, decreased T-cell activation, and decreased angiogenesis (Smedegard and Bjork 1995). Interestingly, sulfapyridine inhibits dihydropteroate, which is essential for folate synthesis; it may, like methotrexate, act as a folate antagonist.

Azathioprine is less commonly used in RA than other DMARDs, and also less frequently used in combination therapy due to side effects such as leukopenia. Azathioprine inhibits purine synthesis and reduces lymphocyte proliferation. Its clinical efficacy has been demonstrated in limited studies (Goebel et al. 1976) but its potential adverse effects as well as the availability of more robust RA treatment limit its use. Some people have a defect in the activity of thiopurine methyltransferase, the enzyme responsible for breaking down azathioprine, and are at risk for cytopenias (Marra et al. 2002). It is currently also used in the treatment of SLE.

HCQ, initially developed as an anti-malarial drug, also has some efficacy in RA, and is discussed in further detail later in this chapter regarding its role in the treatment of systemic lupus erythematosus (SLE).

2.1.2 Biologic DMARDs

Biologic DMARDs (bDMARDs), so named because they are synthesized in living systems, include medications often used in the treatment of patients with rheumatoid arthritis who do not respond to traditional csDMARD therapy. The bDMARDs target specific immune system pathways and their mechanisms of action are more fully understood. The bDMARDs used in RA are monoclonal antibodies or derivatives thereof; as such, they are large molecules that can trigger a host immune response to the drug, and some patients may develop antibodies against bDMARDs that can decrease their efficacy (Wolbink et al. 2006). Currently available bDMARDs in RA include TNF-α inhibitors, IL-6 inhibitors, IL-1 inhibitors, CTLA4 antagonists, and anti-CD20 agents.

Tumor Necrosis Factor-α Inhibitors (TNFi)

TNF-α is a pro-inflammatory cytokine that has been implicated in the pathogenesis of several autoimmune and inflammatory disorders ranging from inflammatory bowel disease and psoriasis to rheumatoid arthritis and other inflammatory arthritides. TNF-α has a variety of biological effects including promoting proliferation and differentiation of inflammatory cells. Its signaling appears to represent a common inflammatory pathway for this heterogeneous group of diseases, thus providing a target for therapy.

TNFi medications were the first bDMARDs used for RA and represented a significant step forward in terms of medication efficacy compared with csDMARDs. Several different TNFi are commercially available at the time of writing, including etanercept (soluble decoy TNF-α receptor, given subcutaneously); adalimumab (fully human anti-TNF-α antibody, given subcutaneously); infliximab (partially humanized TNF-α antibody, given intravenously); golimumab (fully human anti-TNF-α antibody, given subcutaneously or intravenously); and certolizumab (PEGylated Fab fragment of humanized anti-TNF-α antibody, given subcutaneously), as well as their recently-introduced biosimilar counterparts. TNFi work by binding to circulating TNF-α (adalimumab, infliximab, golimumab, certolizumab) or acting as a decoy receptor to block TNF-α's binding sites (etanercept), thus preventing the downstream pro-inflammatory signaling of the cytokine.

TNFi are monoclonal antibodies; because they are large proteins with limited membrane permeability, they are administered parenterally. Of the intravenous TNFi formations, infliximab has an elimination half-life of 8–10 days and is given via weight-based dosing of 3–10 mg/kg every 4–8 weeks, while golimumab has an elimination half-life of 12–14 days and is given via a set weight-based dosing of 2 mg/kg every 8 weeks. Of the subcutaneous TNFi formulations, etanercept has an elimination half-life of 3–5.5 days and is given using a set dose of 50 mg weekly, adalimumab has an elimination half-life of 10–20 days and is given as a 40 mg dose weekly or every other week, golimumab has an elimination half-life of 14 days and

is given using a set dose of, and certolizumab has an elimination half-life of 14 days and is given as 200 mg every 2 weeks or 400 mg every 4 weeks. Certolizumab is also different from other TNFi in that it is PEGylated and lacks an Fc domain, the latter of which is responsible for its inability to cross the placenta in pregnant patients (Mariette et al. 2018).

Several placebo-controlled trials demonstrate the efficacy of TNFi therapy in RA, with improvement in standardized clinical measures such as number of tender and swollen joints. Several studies have demonstrated the efficacy of TNFi in patients with RA: etanercept (Moreland et al. 1997; Weinblatt et al. 1999), infliximab (Maini et al. 1998), adalimumab (Weinblatt et al. 2003), certolizumab pegol (Keystone et al. 2008), and golimumab (Smolen et al. 2009). Differences between individual TNFi in terms of efficacy are difficult to assess as very few head-to-head trials in RA exist, but evidence from one head-to-head trial of adalimumab and certolizumab pegol as well as network meta-analyses of different TNFi in RA suggests that their efficacy is similar in terms of symptoms and reducing joint changes seen on X-ray (Smolen et al. 2016; Singh et al. 2017; Murray et al. 2018).

Due to the crucial role of TNF-α in host defense, TNFi as a class share potential side effects of increased infections including minor infections, serious infections and sepsis, and opportunistic infections, including reactivation of latent tuberculosis infection (LTBI) (Bongartz et al. 2006). Thus, screening for LTBI and attention to vaccination for prevention of infections is essential. Other toxicities associated with TNFi include increased risk of demyelinating polyneuropathy and exacerbation of heart failure, limiting use in patients with prior history of these conditions.

Decreased immune surveillance is also a concern in terms of potential risk of malignancy; TNFi currently have a black box warning due to a potentially increased risk of malignancy observed in clinical studies (Bongartz et al. 2006). However, some registry data have suggested that TNFi are associated with a lower incidence of malignancy (Silva-Fernandez et al. 2016) and other studies have not shown an increase in recurrence of malignancy in RA patients who are treated with TNFi (Raaschou et al. 2018; Dixon et al. 2010). Thus, in certain circumstances including close monitoring in conjunction with an oncologist, TNFi may be safe to use even in patients with a history of prior malignancy.

IL-6 Inhibitors

IL-6 is a pleiotropic cytokine involved in host defense and inflammation; after binding to either the soluble or membrane-bound IL-6 receptor, IL-6 also binds the transmembrane protein, glycoprotein (gp) 130 and transduces a signal via the JAK-STAT pathway (Heinrich et al. 2003; Hennigan and Kavanaugh 2008). Via its effects on the liver, it induces the acute phase response in host defense. IL-6 may contribute to autoimmunity through B-cell modulation and Th17 cell differentiation (Li et al. 2015) and also plays a role in angiogenesis by inducing intracellular adhesion molecules (Nakahara et al. 2003). Excess IL-6 production may, like TNF-α, also activate osteoclasts and contribute to pannus formation and bone and cartilage degradation (Hennigan and Kavanaugh 2008).

Tocilizumab and sarilumab are monoclonal antibodies targeting the IL-6 receptor; they bind both the soluble and membrane-bound forms of the receptor. For RA, tocilizumab is given as a subcutaneous injection of 162 mg weekly or every other week or as an intravenous infusion given via weight-based dosing of 4 mg/kg or 8 mg/kg; patients weighing 100 kg are recommended to receive a flat dose of 800 mg every 4 weeks. Sarilumab is given subcutaneously at a standard dose of 200 mg every other week; this dose may be reduced to 150 mg every other week for any treatment-related laboratory abnormalities.

Both medications are effective in RA patients who do not respond or have a contraindication to methotrexate therapy (Maini et al. 2006; Huizinga et al. 2014); a head-to-head trial for safety and efficacy is currently underway (Emery et al. 2019). In addition to their use in RA, IL-6 inhibitors have also been found to be effective for the treatment of giant cell arteritis (Stone et al. 2017). Siltuximab, which neutralizes IL-6 directly, has not been approved for use in patients with rheumatoid arthritis.

Inhibition of IL-6 has many potential physiologic consequences; thus, the use of anti-IL-6 therapy requires careful monitoring for adverse effects. Infection is among the most common adverse effects; as with TNFi, patients receiving IL-6 inhibitor therapy should be screened for LTBI. In addition, patients taking IL-6 inhibitors should be monitored for gastrointestinal side effects due to the rare incidence of gastrointestinal perforation; it is contraindicated in patients with a history of diverticulitis. Anti-IL-6 therapy also is associated with reversible increases in total cholesterol, increases in liver enzymes, and leukopenia and thus requires regular monitoring of these parameters.

IL-1 Inhibitors

IL-1b is a pro-inflammatory cytokine that is cleaved from its inactive form, Pro-IL-1b, by a multi-protein complex known as the inflammasome. IL-1b, on binding to its receptor, signals via the NK and p38 MAPK pathways to promote expression of other pro-inflammatory cytokines, including IL-6 (O'Neill 2008). Its levels are increased in active rheumatoid arthritis (Eastgate et al. 1988); strong evidence for its involvement in the pathogenesis of inflammatory disorders is provided by the syndrome of inflammation of the skin, bones, and joints that occurs in children with a deficiency of the endogenous IL-1 receptor antagonist and improves with IL-1 inhibition (Aksentijevich et al. 2009). Anakinra (a non-glycosylated form of the human IL-1 receptor that competitively inhibits binding of IL-1α and/or IL-1β) is the only IL-1 antagonist approved for the treatment of rheumatoid arthritis. Given that it is less effective than TNFi (Nixon et al. 2007) and is given as a daily subcutaneous injection, it is not widely used for RA. Canakinumab and rilonacept are IL-1 inhibitors used in other inflammatory disorders and are addressed later in this chapter.

Of the IL-1 inhibitors, anakinra has the shortest elimination half-life of 6 h and is given subcutaneously daily in the treatment of RA, allowing for rapid discontinuation if necessary. Treatment with IL-1 inhibitors, as with other cytokine-blocking therapies, raises a concern for impairment of the innate immune response against pathogens. While URTIs are common with anakinra, opportunistic infections such

as *M. tuberculosis* reactivation are less frequent than with TNFi (Bresnihan et al. 1998). Reversible neutropenia has also been reported (Perrin et al. 2014).

Co-stimulatory Blockade

The CD28:CD80/86 co-stimulatory signal is important to the activation of T cells by antigen-presenting cells and initiation of the T-cell response. Binding of cytotoxic T lymphocyte-associated antigen-4 (CTLA-4) to this receptor, on the other hand, provides an endogenous inhibitory signal that prevents T-cell activation in the adaptive immune response and potentially decreases the inflammatory response in T-cell mediated autoimmune diseases (Alegre et al. 2001). Interestingly, polymorphisms in CTLA-4 have been reported to be associated with autoimmune diseases, including rheumatoid arthritis, in some populations (Lei et al. 2005). This mechanism for suppression of T-cell activation was exploited in the development of the CTLA-4 immunoglobulin fusion protein, abatacept (Salomon and Bluestone 2001).

Abatacept is a fusion protein comprising the extracellular portion of CTLA-4 and the Fc portion of IgG1; by binding CD80 and CD86 on antigen-presenting cells, it blocks the binding of CD28 on T cells and blocks the co-stimulatory signal. Abatacept has been proven effective in rheumatoid arthritis in patients who have not responded to methotrexate (Kremer et al. 2005) or TNFi (Genovese et al. 2005).

The incidence rate of serious infections observed with abatacept treatment is on the lower end compared with other biologics used in RA (Maxwell and Singh 2010), which may be related to its downstream action of inhibiting T-cell activation rather than blocking cytokine production. Overall, the incidence of serious infections observed in a pooled safety analysis was higher than placebo, but those of opportunistic infections such as *M. tuberculosis* were low (Schiff 2011). Malignancy rates for lung cancer and lymphoma were comparable to the baseline rate observed in patients with RA.

B-Cell Antagonists

The contribution of B cells to RA pathogenesis is not completely understood; plasma cells and B cells are found in RA synovium, but the connection between B cells and joint damage has not been fully elucidated and likely involves a combination of B-cell activation, cytokine production, antigen presentation, and antibody production. Interactions between B cells and T cells or other cell types within the synovium also are likely to be contributory (Fox et al. 2010).

Rituximab is a chimeric mouse/human monoclonal antibody against the CD20 protein found on the surface of some B cells, including pre-B cells and mature B cells. The effects of rituximab on CD20+ B cells are thought to involve direct, complement-dependent, and antibody-dependent cytotoxicity (Taylor and Lindorfer 2008) with rapid depletion of peripheral B cells though not mature plasma cells (Leandro 2013). The exact mechanism of rituximab's efficacy in RA is not fully elucidated. Despite its lack of effect on mature plasma cells, rituximab may cause a decrease in autoantibody production by plasmablasts (Leandro 2013), which has been associated with decreased immunoglobulin production in a subset of RA

patients (van Vollenhoven et al. 2015). Rituximab may also lead to depletion of B cells in the synovium (Teng et al. 2007).

The efficacy of rituximab in patients with RA who have not had an adequate response to TNFi or DMARD therapy has been demonstrated in multiple clinical trials (Cohen et al. 2006; Emery et al. 2006). Analysis of pooled registry data suggests that seropositivity for anti-CCP antibodies is associated with a better response to rituximab (Chatzidionysiou et al. 2011), though whether this is mechanistically linked with B-cell function is unknown. Rituximab has also shown efficacy in the treatment of ANCA-associated vasculitis (Stone et al. 2010).

In RA, rituximab is generally given in two doses intravenously every 6 months; of note, the recovery of the peripheral CD20+ B-cell population takes about 6–9 months (Leandro et al. 2006; Roll et al. 2006) As noted above, a subset of patients treated with rituximab develop hypogammaglobulinemia (De La Torre et al. 2012). Due to these factors, several safety considerations should be noted. Infections, including JC virus and *M. tuberculosis*, are relatively rare, but some patients who develop hypogammaglobulinemia after rituximab seem to have a higher risk of infection (Winthrop et al. 2018). Increased risk of malignancy has not been observed in long-term studies of rituximab treatment compared to expected rates in unexposed patients (Winthrop et al. 2018; Boleto et al. 2018).

2.1.3 tsDMARDs

TsDMARDs are small molecules that interfere with intracellular signaling and cytokine production. Currently available tsDMARDs used in the treatment of RA are inhibitors of JAK isotypes, though inhibitors of SYK are also being studied.

JAK Inhibitors

JAKs are intracellular enzymes that transduce signals from certain membrane receptors binding to ligand. They then form homo- or heterodimers which autophosphorylate, then phosphorylate other intracellular proteins, including signal transducer and activator of transcription (STAT) DNA binding proteins. This signal transduction is involved in many physiologic processes, including the function and signaling of hormones, growth factors, and cytokines (Choy 2019). There are four JAK isotypes in humans: JAK1, JAK2, JAK3, and tyrosine kinase 2 (O'Shea et al. 2015). JAK inhibitors are small molecules that inhibit the JAK-STAT pathway and thereby reduce downstream cytokine production (Jamilloux et al. 2019); different JAK inhibitors likely decrease the signaling of multiple different cytokines. Complete inhibition of JAK isotypes is detrimental because of their widespread effects; prior studies in humans and animals have shown that mutations or deficiency of JAK isotypes may be lethal or lead to immunodeficiency (Thomis et al. 1995; Macchi et al. 1995; Parganas et al. 1998; Rodig et al. 1998). Instead, the JAK inhibitors used in RA reversibly inhibit signaling and cytokine function (Choy 2019).

Baricitinib, tofacitinib, and upadacitinib are currently available for the treatment of rheumatoid arthritis; other JAK inhibitors are under investigation. All three are oral and have shorter elimination half-lives than the monoclonal antibody bDMARDs, ranging from 3 to 16 h (Taylor 2019). The JAK inhibitors have different

specificities for JAK isotypes: tofacitinib targets JAK1 and JAK3, baricitinib targets JAK1, JAK2, and, to a lesser extent, Tyk2, and upadacitinib is selective against JAK1 (Parmentier et al. 2018; Clark et al. 2014). RCTs have demonstrated that all three are effective in patients with RA with an inadequate response to TNFi and methotrexate: tofacitinib (Fleischmann et al. 2012); baricitinib (Genovese et al. 2016a, b; Dougados et al. 2017); upadacitinib (Kremer et al. 2016). A network meta-analysis of all three JAK inhibitors did not identify statistically significant differences in efficacy for patients with RA, though upadacitinib had a numerically higher efficacy with respect to clinical symptoms and achievement of remission.

In addition to monitoring for infection due to concerns regarding suppression of normal immune system responses, JAK inhibitors share risks for specific complications during therapy, including reactivation of herpes zoster (Winthrop 2017); vaccination to prevent herpes zoster is recommended. Use of JAK inhibitors also may be associated with increased risk of thrombosis. A safety review of postmarketing adverse event reports suggested an increase in risk of pulmonary thrombosis though not pulmonary embolism of deep venous thrombosis with tofacitinib (Verden et al. 2018); whether this is higher than the baseline rate of thrombosis in RA patients is not yet known, so cautious monitoring is advisable.

2.2 Seronegative Spondyloarthritis

Seronegative spondyloarthritis (SpA), so named because the rheumatoid factor is negative, is a term referring to group of diseases loosely linked by shared clinical characteristics of inflammatory back pain, sacroiliitis, asymmetric, oligoarticular joint inflammation, enthesitis, ocular inflammation, inflammation of the gastrointestinal tract, and rashes; some of them are associated with the human leukocyte antigen B27 (HLA-B27) allele. They include ankylosing spondylitis (AS), reactive arthritis, psoriatic arthritis (PsA), arthritis associated with inflammatory bowel disease, and other diseases such as uveitis. The term SpA is used here to refer to the group, unless otherwise noted in order to discuss disease-based differences in treatments.

AS is a prototypical SpA in which the subchondral bone marrow at margins of the vertebrae become replaced by granulation-tissue and then forms syndesmophytes (Bleil et al. 2016). As this ossification progresses, the vertebrae become fused, leading to the typical appearance of "bamboo spine" on X-ray. PsA can also cause bony ankylosis but is most prominently associated with enthesitis, dactylitis, and the scaly red rash known as psoriasis. Other types of SpA can also present with similar peripheral arthritis or axial symptoms.

While a discussion of the pathogenesis of each of these disorders separately is beyond the scope of this chapter, possible mechanisms of the classes of medications used in their treatment are reviewed; many of these medications overlap with those used in the treatment of RA.

2.2.1 csDMARDs

For SpA associated with joint and lower back pain, non-steroidal anti-inflammatory drugs (NSAIDs) are used for symptomatic relief; beyond this, methotrexate and sulfasalazine, reviewed previously in this chapter in the treatment of rheumatoid arthritis, are used for peripheral joint pain and inflammation (Taylor 2019). Methotrexate is commonly used for psoriasis and joint symptoms in PsA (Mease et al. 2019), but has not been found to be effective for AS, including peripheral arthritis and enthesitis (Chen et al. 2006).

2.2.2 bDMARDs

Typically, bDMARDs are the mainstays of therapy for SpA due to inconsistencies in patients' responses to csDMARDs, used to improve skin and joint symptoms in PsA as well as pain and function in AS with approximately comparable efficacy (Deodhar et al. 2020; Ruyssen-Witrand et al. 2020). TNFi are most commonly used, while IL-1 and IL-6 inhibitors have not proven particularly effective. All five of the TNFi reviewed above in the treatment of RA are also used in SpA; their mechanism of action is similar in terms of inhibiting downstream signaling of TNF-α, which, as in RA, promotes osteoclastogenesis and formation of erosions. In addition, in SpA, differentiation and propagation of helper T cells along Th1 and Th17 cell lineages lead to the release of pro-inflammatory cytokines, including TNF-α; the cytokines involved in this process provide additional targets for therapy.

IL-17 Inhibitors

Th17 cells produce IL-22 as well as the pro-inflammatory cytokines IL-17A and IL-17F, which are thought to be involved in recruitment of neutrophils and macrophages as well as promotion of bony destruction via matrix metalloproteinases. The currently available IL-17 inhibitors used for treatment of SpA include secukinumab, a human IgG1 monoclonal antibody that binds to IL-17A, brodalumab, a human IgG2 monoclonal antibody that binds to the IL-17 receptor-A (IL-17R), and ixekizumab, a humanized IgG4 monoclonal antibody that binds to IL-17A. Bimekizumab, an IgG1k humanized monoclonal antibody that binds to IL-17A and IL-17F, is approved for the treatment of psoriasis and is under investigation for ankylosing spondylitis and psoriatic arthritis.

Several randomized, double-blind, placebo-controlled trials have demonstrated the efficacy of IL-17 inhibitors in the treatment of SpA. Secukinumab has efficacy for joint and skin symptoms in PsA as well as axial symptoms in AS (Mease et al. 2015; McInnes et al. 2015; Baeten et al. 2015). Similarly, ixekizumab is also useful for the treatment of PsA and AS. Head-to-head studies demonstrated its superiority compared to adalimumab (Mease et al. 2020) in PSA and comparability to adalimumab in AS (van der Heijde et al. 2018). Brodalumab has been shown to be effective in clinical trials in the treatment of psoriatic arthritis (Mease et al. 2014); studies are ongoing in AS 24918373 (Wei et al. 2019).

All three IL-17 inhibitors are given via subcutaneous injection using a loading dose phase followed by maintenance therapy. Secukinumab is given as a

maintenance dose of 150 mg dose every month, but can be increased to 300 mg every month for patients with moderate to severe psoriasis (Mease et al. 2018).

Adverse effects of IL-17 inhibitors, as with other bDMARDs, include infections, including respiratory infections such as nasopharyngitis. A slight increase in fungal infections with different *Candida* species has been seen throughout clinical trials with IL-17 inhibitors, perhaps due to the prominent role that IL-17 plays in muco-cutaneous immunity; *Candida* infections were reported in 4.0%, 1.7%, and 3.3% of patients treated with brodalumab, secukinumab, and ixekizumab, respectively, compared to 0.3%, 2.3% and 0.8% of patients who received placebo, ustekinumab, or etanercept, respectively (Saunte et al. 2017). As such, patients receiving IL-17 inhibitors should be monitored for fungal infection.

Another non-infectious adverse event purported to be associated with IL-17 inhibitor therapy is inflammatory bowel disease; published clinical trials reported exacerbations of gastrointestinal symptoms in patients treated for Crohn's disease with secukinumab (Hueber et al. 2012). However, a pooled analysis of trials of secukinumab for psoriasis, PsA, and AS showed low numbers of inflammatory bowel disease events and no increase over time with continued exposure (Schreiber et al. 2019).

Due to six reported suicides in the clinical trials of brodalumab (31024633), an association between IL-17 inhibitors and suicide has been proposed, but has not been seen with secukinumab or ixekizumab or borne out in a long-term study of brodalumab (Lebwohl et al. 2019). Interestingly, Th17 cells and IL-17 can have both pro-tumor and anti-tumor effects (Murugaiyan and Saha 2009) and the effects of IL-17 inhibitors on malignancy are not known.

IL-12/23 Inhibitors

IL-23 acts upstream of IL-17 by promoting Th17 cell differentiation and release of IL-17; IL-12, on the other hand, promotes Th1 differentiation and release of TNF-a. Therapies targeting IL-12 and IL-23 are effective in the treatment of PsA but not AS, suggesting a difference in the pathogenesis of these two types of SpA.

Ustekinumab, a monoclonal antibody against common p40 subunit of IL-12 and IL-23, is effective in PsA (23769296, 24482301), with improvement in both skin and joint symptoms as well as progression of damage seen on X-ray. IL-23-specific agents targeting the p19 subunit unique to IL-23 have also been investigated as treatment for PsA. At the time of writing, two of these, guselkumab and risankizumab, are FDA-approved for its treatment. Because of their relatively recent introduction as therapeutic agents, the comparative of IL-23 inhibitors compared to other treatments for PsA have not been established.

Of these bDMARDs targeting the IL-12/23 axis, risankizumab and tildrakizumab are humanized IgG1 and IgG1 k constructs, while guselkumab and ustekinumab are fully human monoclonal antibodies. All four agents are given after a loading phase at weeks 0–4 followed by maintenance therapy every 8–12 weeks.

Pooled safety data in psoriasis suggests that IL-23 inhibitors are comparable to other biologics in terms of safety, with an increase in nasopharyngitis (Bai et al.

2019), while ustekinumab is associated with fewer serious infections than IL-17 inhibitors and TNFi.

2.2.3 Targeted Synthetic DMARDs

JAKi

JAKi were reviewed previously in this chapter in treatment of rheumatoid arthritis, but have also shown promise in the treatment of SpA, possibly due to similar inhibition of cytokine pathways involved in inflammation. Tofacitinib has improved skin and joint symptoms in clinical trials of psoriatic arthritis patients who have previously had inadequate relief of symptoms with methotrexate or TNFi (Gladman et al. 2017; Mease et al. 2017). In addition, upadacitinib was studied in patients with AS who did not have improvement with NSAID; preliminary data showed evidence of improved symptoms at 3 months (van der Heijde et al. 2019).

Phosphodiesterase 4 Inhibitors

Phosphodiesterase 4 inhibitors catalyze the degradation of cyclic adenosine monophosphate, which in turn acts to regulate intracellular signaling in a variety of pathways, including downstream production of pro-inflammatory cytokines. PDE4 mRNA has been found to be elevated in patients with psoriasis compared to healthy controls, and apremilast, a PDE4 inhibitor, reduced production of such as TNF-a, interferon (IFN)-g, and IL-17 and increased production of the anti-inflammatory cytokines such as IL-10 in vitro (Schafer et al. 2016).

Apremilast has been shown to have efficacy in patients with PsA in several clinical trials, for both psoriasis and arthritis and in patients who had not previously been treated as well as those who had not had improvement with csDMARDs and bDMARDs (Kavanaugh et al. 2014; Wells et al. 2018). Its efficacy in PsA is thought to be lower than that of most bDMARDs (Ruyssen-Witrand et al. 2020), and so it is generally attempted in patients with PsA who have contraindications or who have not yet progressed to bDMARD therapy. PDE4 inhibitors have also been investigated in other immune-mediated conditions such as SLE and RA without success thus far; apremilast was studied for the treatment of ankylosing spondylitis but did not show any improvement in axial symptoms (Pathan et al. 2013).

Apremilast is given orally, most commonly twice daily; its elimination half-life is 6–9 h. Although trials have reported an increase in minor infections, pooled safety data have shown low rates of serious infections (Crowley et al. 2017). Its most common side effects include diarrhea and nausea (24595547). Because of increased reports of depression with apremilast use compared to placebo in clinical trials, caution is advised in treating patients with mood disorders; however, a cohort study comparing apremilast to other therapies for PsA found that users of apremilast had similar rates depression and slightly higher rates of anxiety (Vasilakis-Scaramozza et al. 2020).

2.3 Systemic Lupus Erythematosus

SLE is an autoimmune disease with protean manifestations including some similar to RA and SpA (arthritis, bowel inflammation, and uveitis) and many others that are unique (malar rash, alopecia, and glomerulonephritis). SLE is characterized by serologic abnormalities such as autoantibody production (particularly anti-double stranded DNA) and low complement levels, as well as leukopenia, anemia, and thrombocytopenia. The pathogenesis of SLE is as yet unknown; as with the other autoimmune diseases discussed in this chapter, it likely involves defects of multiple arms of the immune system.

The medications used in the treatment of SLE may provide some insights as to its pathogenesis. Many immunomodulatory medications used off-label for the management of lupus symptoms are also used in other conditions. In contrast to the extensive progress made in the treatment of RA through the advent of bDMARDs in the 1990s and 2000s, belimumab, as the first biologic approved for SLE in 2011, was also the first medication approved by the FDA for SLE since 1955, when hydroxychloroquine and corticosteroids were approved.

Although SLE has many manifestations, the next section focuses mostly on agents used for patients' cutaneous and musculoskeletal symptoms as well as renal involvement, i.e. lupus nephritis.

2.3.1 csDMARDs

The mainstay of therapy for SLE is the anti-malarial HCQ, mentioned previously as it is also used in the treatment of RA. Similarly, methotrexate and azathioprine, discussed above in the treatment of RA, are used mostly for the cutaneous and articular symptoms of SLE and sometimes for organ system involvement. Other antiproliferative agents and calcineurin inhibitors are discussed elsewhere in this textbook in their roles in conditions such as malignancy and prevention of transplant rejection.

Anti-malarial Agents

Initially developed as an anti-malarial drug, HCQ has not proven to be immunosuppressive per se; in fact, its mechanism of action in autoimmune conditions is not well understood. HCQ and other anti-malarial agents are thought to increase lysosomal pH (Fox 1993), potentially interfering with autoantigen presentation, as well as inhibit Toll-like receptor 9 signaling (Kuznik et al. 2011), thereby decreasing dendritic cell activation and T-cell-derived inflammation. While its use in the treatment of COVID-19 has been controversial in part due to the frequency of adverse effects such as prolongation of the QT interval (Mercuro et al. 2020), it is generally well tolerated in the treatment of autoimmune disease, with common side effects including nausea and skin discoloration. Retinal toxicity is infrequent with doses of HCQ < 5 mg/kg/day, but cumulative dose is a risk factor (Melles and Marmor 2014), and regular ophthalmologic screening including visual field examination is recommended for patients on HCQ. Other anti-malarial agents such as chloroquine and quinacrine are also used, primarily for chronic and subacute

cutaneous lupus erythematosus; while retinopathy is reported with chloroquine use as well, it is not frequently seen with quinacrine (Mittal et al. 2018).

Cyclophosphamide

Cyclophosphamide (CYC) is an alkylating agent and potent immunosuppressant; in rheumatic disease, it is used primarily in severe manifestations of SLE and vasculitis, including small vessel vasculitis. It can be administered both orally as a daily dose and intravenously every 2–4 weeks, and is rapidly absorbed and metabolized into active alkylating compounds in the liver (Takada et al. 2001). It is postulated to work via induction of DNA damage as well as reduction in T- and B- lymphocyte number and function (3259286).

For lupus nephritis, CYC is usually given intravenously in the context of induction therapy before switching to a different medication for maintenance, approximately 500–1,000 mg/m^2 in monthly or biweekly regimens. Different dosing protocols have been tried in order to reduce cumulative exposure to CYC (Austin 3rd et al. 1986; Houssiau et al. 2002), with comparable short- and long-term results (Houssiau et al. 2002, 2010). Limiting toxicities include cytopenias, such that blood counts should be monitored during therapy in order to adjust CYC dose, as well as gastrointestinal side effects, gonadal toxicity, increased risk of infection, and hemorrhagic cystitis with a possible link to bladder cancer (Monach et al. 2010). The latter adverse effect has led some to suggest the use of mesna or intravenous hydration in conjunction with CYC therapy, though evidence is lacking (Monach et al. 2010).

Mycophenolate Mofetil

In contrast to HCQ and other agents used for non-organ-threatening manifestations of SLE, mycophenolate mofetil (MMF) is frequently used in SLE for renal manifestations, i.e. lupus nephritis. MMF is a prodrug of mycophenolic acid; both are used in clinical practice, though MMF is more common. Mycophenolic acid reduces T- and B-cell proliferation by inhibiting inosine-5′-monophosphate dehydrogenase (IMPDH) in lymphocytes, thus decreasing guanosine nucleoside synthesis (Allison and Eugui 2000) and decreasing T-cell mediated immunity as well as antibody production. This antiproliferative effect has also been harnessed for the prevention of renal allograft rejection. In addition, MMF interferes with the expression and glycosylation of adhesion molecules that facilitate recruitment of monocytes and lymphocytes to areas of inflammation (Allison 2005).

MMF's efficacy has been studied in induction of remission in lupus nephritis and shown to be comparable to IV CYC in terms of response rates (Appel et al. 2009; Ginzler et al. 2005) while for maintenance therapy, MMF was found to be superior to azathioprine (Dooley et al. 2011). For both indications, MMF is generally used orally at 2–3 g daily in divided doses. Dosing is sometimes limited by its side effects, including diarrhea and leukopenia, and an increase in viral infections has also been reported with MMF use (Appel et al. 2009).

Calcineurin Inhibitors

Calcineurin inhibitors (CNI) have long been used in the treatment of SLE and lupus nephritis; they inhibit T-cell activation via inhibition of signal transduction by calcineurin, a calcium- and calmodulin-dependent phosphatase, and also have an antiproteinuric effect (Mok 2017). They have a limited therapeutic window due to toxicities of hypertension, dyslipidemia, hyperglycemia, nephrotoxicity, and neurotoxicity (tremors) (Mok 2017) and require monitoring of serum drug levels.

Tacrolimus and cyclosporine are older CNI; of the two, tacrolimus has been more frequently used in SLE due to its more favorable side effect profile. While tacrolimus has shown promise alone and in combination in induction therapy of lupus nephritis, studies are limited by outcomes studied and short duration of follow-up (Bao et al. 2008; Mok et al. 2016). Voclosporin is a newer CNI with reportedly fewer metabolic side effects than cyclosporine or tacrolimus in renal transplant recipients (Busque et al. 2011); though studies in lupus nephritis have not yet been published, preliminary data suggest that it is effective in remission induction (Arriens et al. 2020).

2.3.2 bDMARDs

Belimumab

B-cell activating factor (BAFF), otherwise known as B-lymphocyte stimulator (BLyS), is a member of the TNF superfamily of cytokines and promotes B-cell survival and maturation (Shin et al. 2018). It is thought to be involved in SLE by promoting the survival of autoreactive B cells and subsequent autoantibody production; BAFF levels are elevated in patients with SLE and correlate to autoantibody titers (Cheema et al. 2001; Zhang et al. 2001). Belimumab is a human monoclonal IgG1-lambda antibody against the soluble form of BAFF; it has been shown to be effective in nonrenal SLE based on improvement in disease activity indices as well as in complement and autoantibody levels (Stohl et al. 2012; Furie et al. 2011). Belimumab is also being evaluated for lupus nephritis due to a finding of reduced renal flares on post hoc analysis compared to placebo. Other BAFF inhibitors have been studied for SLE, but none are commonly used in practice; atacicept was not found to be more effective than placebo in prevention of lupus flares (Isenberg et al. 2015).

Belimumab is used either intravenously every month or subcutaneously every week. Like the other biologics discussed in this chapter, its use in clinical trials was associated with an increase in headache, nausea, and minor infections (Merrill et al. 2012). More deaths were reported in clinical trials with belimumab use compared to placebo, as well as more episodes of depression. Though the mechanism for these changes is unknown, caution is advised for patients with a history of depression.

Anifrolumab

Type I IFN has long been thought to be involved in the pathogenesis of SLE, although until recently it has not been successfully targeted in therapeutic trials. Indirect evidence supports the role of type I IFN and the innate immune system (along with, as discussed above, B cells and humoral immunity) in SLE: differential

methylation of genes induced by type I IFN has been noted in people with SLE (Coit et al. 2013) and genetic variants causing increased type I IFN activity are a risk factor for developing SLE (Niewold et al. 2007). Anifrolumab is a fully human IgG1κ monoclonal antibody against IFN-a receptor 1; it blocks IFN signaling as well as autoamplification of IFN production (Riggs et al. 2018).

Anifrolumab, given intravenously once a month, has been studied in nonrenal SLE with mixed, though promising, results in terms of improvement in disease activity and reduction of steroid dose (Morand et al. 2020) and is under investigation for lupus nephritis. As it is a relatively new bDMARD, little is known about its long-term safety, but it use in clinical trials is associated with increased frequency of herpes zoster and bronchitis (Morand et al. 2020).

2.4 Autoinflammatory Disorders

In contrast to autoimmune disorders such as SLE and RA in which both mediators of both innate and adaptive immunity promote inflammation, autoinflammatory disorders primarily involve dysregulation of the innate immune system. Prototypical autoinflammatory disorders include monogenic periodic fever syndromes such as familial Mediterranean fever (FMF), periodic fever with aphthous stomatitis, pharyngitis, and cervical adenopathy syndrome (PFAPA), and TNF-α-receptor-associated periodic fever syndrome (TRAPS), as well as multifactorial pyogenic diseases such as systemic onset juvenile idiopathic arthritis (SJIA) and Behçet's disease. Crystal-induced arthropathies such as gout have also sometimes been included in this categorization. This group of disorders is linked by common symptoms of fever, inflammatory arthritis, dermo-hypodermitis, and in some cases gastrointestinal symptoms such as abdominal pain and diarrhea. Most are relatively rare; SJIA, which is discussed in more detail, has an incidence of 0.4–0.8 per 100,000 (Gurion et al. 2012).

Generally, autoinflammatory disorders are not associated with detectable autoantibodies or autoreactive T cells, but rather macrophage- and monocyte-driven tissue inflammation and damage (Hedrich 2016). Pattern recognition receptors can recognize these (host) damage-associated molecular patterns. In particular, activation of the Nod-like receptor (NLR) through mutations in NLR genes in different autoinflammatory disorders can lead to production of innate pro-inflammatory cytokines such as IL-1β; these in turn are potential targets in the treatment of these diseases (Doria et al. 2012).

2.4.1 Colchicine

Colchicine has long been known to bind to tubulins and block the polymerization and formation of microtubules. It has additional effects on the immune system, including inhibition of the function of neutrophils, macrophages, and the NALP3 inflammasome. The mechanism of colchicine's suppression of the NALP3 inflammasome and innate immune responses is not fully understood, but may also be related to the inflammasome's dependence on microtubule formation.

Colchicine's therapeutic window is narrow due to its common side effects; at prophylactic daily doses used in periodic fever syndromes as well as acute doses used in gout, it can cause gastrointestinal upset and diarrhea (Moreira et al. 2017). Use of colchicine can also cause myelosuppression with cytopenias; as it is metabolized by cytochrome P450 3A4 (CYP3A4) and excreted via the P-glycoprotein (P-gp) transport system, caution must be used in patients with renal or hepatic impairment, especially with concurrent use of CYP 3A4 inhibitors or P-gp inhibitors (Leung et al. 2015).

2.4.2 IL-1 Inhibitors

Though other treatments, including some of the bDMARDs and csDMARDs discussed earlier in the treatment of RA are used for juvenile idiopathic arthritis (JIA), the management of sJIA and other autoinflammatory disorders is slightly different, commonly relying on glucocorticoids and IL-1 inhibition. Anakinra, also used in the treatment of RA, canakinumab (a human monoclonal anti-IL-1β antibody), and rilonacept (a human IL-1R1/IL-1R accessory protein fused to the Fc portion of IgG1) are potent targeted inhibitors of IL-1α and/or IL-1β signaling. All are given subcutaneously; elimination half-lives are significantly longer for canakinumab (26 days) and rilonacept (7 days) compared to anakinra (4–6 h), and thus they are administered at monthly or weekly intervals respectively, compared to daily with anakinra (Jesus and Goldbach-Mansky 2014).

Given the relative rarity of these illnesses and their predominance in children, the breadth of clinical trials of IL-1 inhibitors in autoinflammatory disorders is limited. In SJIA, anakinra (Quartier et al. 2011), rilonacept (Lovell et al. 2013; Ilowite et al. 2014), and canakinumab (Ruperto et al. 2012) have been shown to improve joint symptoms in clinical trials as well as reduce fever and allow for tapering of glucocorticoid dose. The latter effect is especially important due to its implications in ameliorating steroid-induced growth reduction in children.

The main associated adverse effects with IL-1 inhibitors are injection site reactions, particularly in the use of daily injections of anakinra (Kaiser et al. 2012), as well as an increase in non-serious infections. Thus far, adverse effects in the pediatric population do not seem to be markedly different from those in the adults.

2.5 Systemic Vasculitis

Systemic vasculitis is a broad and complex category of rheumatic diseases; some types of vasculitis are primary while others are related to other conditions such as RA or SLE. Treatments for systemic vasculitis are thus variable based on the underlying condition and its manifestations; broader categorization is thus difficult. Methotrexate, for example, is frequently used in the treatment of ANCA-associated vasculitis confined to the upper respiratory tract, while CYC and rituximab are used in severe organ-threatening ANCA vasculitis (Stone et al. 2010). MMF is used for organ-threatening vasculitis including glomerulonephritis secondary to lupus, as reviewed

earlier. Tocilizumab is used in the treatment of giant cell arteritis (Stone et al. 2017), which otherwise is commonly treated with glucocorticoids. These agents are addressed extensively earlier in this chapter and will not be treated separately here.

3 Conclusions

The treatment of rheumatologic conditions goes hand in hand with understanding of immunology and pharmacology. People with rheumatic diseases may experience symptoms and side effects from a combination of autoimmunity, inflammation, and immunodeficiency, in addition to which immunosuppressive therapy adds a layer of complexity. We are beginning to understand the ways in which rheumatic diseases and their treatments may increase patients' risk of developing malignancies, bacterial, viral, and fungal infections, and cardiovascular complications, as discussed with respect to medication side effects in this chapter. In reality, much more information from safety analyses needs to be evaluated to understand these nuances over the long term.

Consideration must also be given to the fact that people with rheumatic diseases often live with conditions and may be treated with medications over the course of months to decades. Some medications may lose efficacy over the course of years of therapy; monoclonal antibodies can provoke the development of human anti-chimeric or human anti-human antibodies (HACAs and HAHAs, respectively), requiring an increase in dose or a change in medication. Rheumatic disease patients, including children, elderly persons, and people of reproductive age, may take medications throughout their lifespan; not only must we understand long-term risks and safety profiles, but also how therapeutic choices and their consequences may change during different stages of the life cycle.

While our understanding of the pathogenesis of rheumatologic conditions is not yet complete, the advent of immunomodulatory and immunosuppressive medications has represented a breakthrough for people with rheumatic diseases in terms of improving their symptoms and quality of life. A variety of monoclonal antibodies and small molecule synthetic agents with new targets are being studied in RA and SLE/lupus nephritis; whether their use translates to better understanding of disease pathogenesis remains to be seen. That many people have conditions that are "refractory" to the therapies reviewed above challenges the scientific community to continue to explore and develop new therapies in order to provide more treatment options for patients and further insight into their conditions.

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Immune Suppression in Allogeneic Hematopoietic Stem Cell Transplantation

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Abstract

Allogeneic hematopoietic stem cell transplantation (allo-HSCT) is a curative treatment for high-risk hematologic disorders. There are multiple immune-mediated complications following allo-HSCT that are prevented and/or treated by immunosuppressive agents. Principal among these immune-mediated complications is acute graft-versus-host disease (aGVHD), which occurs when the new donor immune system targets host tissue antigens. The immunobiology of aGVHD is complex and involves all aspects of the immune system. Due to the risk of aGVHD, immunosuppressive aGVHD prophylaxis is required for nearly all allogeneic HSCT recipients. Despite prophylaxis, aGVHD remains a major cause of nonrelapse mortality. Here, we discuss the clinical features of aGVHD, the immunobiology of aGVHD, the immunosuppressive therapies used to prevent and treat aGVHD, how to mitigate the side effects of these immunosuppressive therapies, and what additional immune-mediated post-allo-HSCT complications are also treated with immunosuppression.

Keywords

Acute graft-versus-host disease · Allogeneic hematopoietic stem cell transplantation · Immune suppression

1 Introduction

Hematopoietic stem cell transplantation (HSCT) is a curative modality for high-risk malignancies, hematologic disorders, immunologic disorders, and metabolic disorders (Hołowiecki 2008). Fundamentally, HSCT results in a complete or partial replacement of the hematopoietic system. The procedure is performed by first conditioning the recipient with chemotherapy and/or total body irradiation followed by the infusion of donor HSCs. Conditioning serves to make physical space in the recipient bone marrow for the new HSC graft and to suppress the recipient's immune

system to prevent graft rejection. Following stem cell engraftment, the donor graft repopulates the hematopoietic and immunologic compartments.

There are two main categories of HSCT: autologous and allogeneic. In autologous HSCT, the hematopoietic compartment is rescued with a cryopreserved autologous HSC product harvested from the recipient prior to conditioning. Autologous HSCT is typically used to reconstitute hematopoiesis following consolidative, high-dose, myeloablative chemotherapy regimens for lymphomas and various solid tumors thereby overcoming the hematopoietic dose-limiting toxicity of these consolidative regimens.

In contrast to autologous HSCT, the stem cell graft in allogeneic HSCT is derived from a different person than the recipient, which makes allogeneic HSCT useful for treating hematologic, immunologic, and metabolic disorders. Because the graft donor and recipient are different people in allogeneic HSCT, polymorphic antigens will differ between the donor and recipient. These polymorphic antigens are recognized by donor allogeneic T cells, which are the primary drivers of alloimmunity. Alloimmune reactions are beneficial when the donor alloimmune response is directed against polymorphic antigens present on tumor cells. This antitumor response is termed the graft-versus-tumor (GVT) effect and represents one of the first immunologic therapies for cancer. However, alloimmune reactions can also be directed against polymorphic allogeneic antigens present on host tissues resulting in acute graft-versus-host disease (aGVHD). These activated allogenic antigen-responsive T cells then drive the immune-mediated damage of the main aGVHD target organs in the recipient, namely the skin, liver, and gastrointestinal (GI) tract (Ferrara et al. 2009). Due to the risk of aGVHD, nearly all allogeneic HSCT recipients receive aGVHD prophylaxis with immunosuppressive therapies. Despite prophylaxis, aGVHD occurs in 30–50% of patients and remains a major life-threatening complication of allogeneic HSCT (Ferrara et al. 2009). Herein, we discuss the pathophysiology of aGVHD, the immunosuppressive therapies used to prevent and treat aGVHD, and how best to mitigate the myriad off-target and on-target side effects of these therapies, including infection and relapse.

2 Clinical Features of aGVHD

The main organs affected by aGVHD are the skin, liver, and GI tract. In rare instances, the lungs, central nervous system, and retinas are also affected (Zeiser and Blazar 2017a). Acute GVHD typically manifests within the first 100 days after transplantation; however, it can occur later (Zeiser and Blazar 2017a). The risk of aGVHD is increased by HLA-mismatched grafts, advanced age of the recipient or donor, male recipients of female donors, unmanipulated peripheral blood stem cell grafts relative to bone marrow or umbilical cord blood grafts, and with myeloablative conditioning regimens relative to reduced intensity regimens (Zeiser and Blazar 2017a; Jagasia et al. 2012; Flowers et al. 2011; Hahn et al. 2008).

The skin is typically the first organ affected by aGVHD (Ferrara et al. 2009). Signs of skin aGVHD include an erythematous maculo-papular rash that can

advance to blisters and ulceration (Ferrara et al. 2009; Zeiser and Blazar 2017a). Early skin aGVHD has a predilection for the palms, soles, ears, neck, and dorsal surfaces of the extremities and malar regions (Ferrara et al. 2009; Zeiser and Blazar 2017a). Histology of skin aGVHD typically reveals apoptosis at the basal membrane of the epidermal layer, dyskeratosis, exocytosis of lymphocytes, satellite lymphocytes adjacent to dyskeratotic epidermal keratinocytes, and perivascular lymphocytic infiltration in the dermis (Zeiser and Blazar 2017a). These histopathological findings often overlap with those of drug reactions and infectious etiologies, thereby limiting the usefulness of skin biopsy for the diagnosis of cutaneous aGVHD (Haimes et al. 2019; Zhou et al. 2000). Upper GI aGVHD typically manifests with nausea, weight loss, and anorexia (Ferrara et al. 2009; Zeiser and Blazar 2017a). Patchy ulcerations and flattening of surface epithelium are typically seen on histopathology (Zeiser and Blazar 2017a). Lower GI aGVHD manifests as watery and/or bloody diarrhea with or without crampy abdominal pain (Ferrara et al. 2009; Zeiser and Blazar 2017a). Apoptotic bodies and abscesses in the epithelial crypts are diagnostic on histopathology of endoscopic biopsies (Ferrara et al. 2009; Zeiser and Blazar 2017a). Liver aGVHD clinically manifests with elevated total bilirubin with or without jaundice (Ferrara et al. 2009; Zeiser and Blazar 2017a). Pathology is notable for lymphocytic infiltration near port veins and bile ducts with bile duct loss occurring in advanced lesions (Ferrara et al. 2009; Zeiser and Blazar 2017a).

The severity of aGVHD is staged within each of the primary target organs: skin, liver, and gut (Glucksberg et al. 1974; Przepiorka et al. 1995). These stages are then combined into an overall grade (Glucksberg et al. 1974; Przepiorka et al. 1995). The skin is staged from 0 to 4 based on the percent of body surface area involvement (stage 0, no rash; stage 1, rash <25% body surface area (BSA); stage 2, 25–50% BSA; stage 3, generalized erythroderma or rash >50% BSA; stage 4, generalized erythroderma plus bullous formation and desquamation >5% BSA). Liver GVHD is staged based on the serum total bilirubin level (stage 0, <2 mg/dL; stage 1, 2–3 mg/dL; stage 2, 3.1–6 mg/dL; stage 3, 6.1–15 mg/dL; stage 4, >15 mg/dL). The GI tract is staged based on the volume of stool output per day in adults (patients ≥50 kg in weight), or stool output per kilogram bodyweight in children (stage 0, <500 mL/day or <30 mL/kg; stage 1, >500 mL/day or >30 mL/kg; stage 2, >1,000 mL/day or >60 mL/kg; stage 3, >1,500 mL/day or >90 mL/kg; stage 4, severe abdominal pain with or without ileus, or grossly bloody stool, regardless of stool volume). Isolated acute upper GI GVHD confirmed by upper GI biopsy is considered stage 1.

The Glucksberg Scale is the most widely used system for grading aGVHD and reflects the fact that the GI tract is the target organ most associated with nonrelapse mortality (Przepiorka et al. 1995; MacMillan et al. 2020). Mild, grade I acute GVHD, consists of stage 1 or 2 skin involvement without liver or GI involvement. Moderate, grade II GVHD, consists of stage 3 skin involvement or stage 1 liver or GI involvement. Grade III, severe, acute GVHD consists of stage 0–3 skin, with stage 2–3 liver or GI involvement. Finally, grade IV, very severe and life-threatening acute GVHD, consists of stage 4 skin, liver or GI involvement. Acute GVHD occurs in 30–50% of all allogeneic HSCT recipients and is severe (grade III-IV) in approximately 15% (Zeiser and Blazar 2017a). While the Glucksberg Scale is widely

employed clinically, recent studies have found that it does not optimally predict outcomes. Newer algorithms using clinical criteria or biomarkers are showing promise and are being explored as potentially useful early parameters to intervene upon in order to improve treatment response and survival in high-risk aGVHD (MacMillan et al. 2020; Levine et al. 2015; Hartwell et al. 2017; Major-Monfried et al. 2018; Gergoudis et al. 2020).

3 Influence of Donor Graft, MHC Matching, and Conditioning on aGVHD

Acute GVHD is understood as a donor allogeneic T cell-dependent response to disparate histocompatibility antigens in an immunocompromised host. The recipient must be immunocompromised, typically as a result of conditioning, or the host immune system will prevent the donor allogeneic T cells from engrafting and responding to these disparate antigens. Genetic polymorphisms between the donor and recipient are responsible for these disparate antigens, of which the histocompatibility antigens are the most influential. Histocompatibility antigens are designated as either major (MHC) or minor (miHA) based on their degree of immunogenicity. The MHC complex, also referred to as the human leukocyte antigen (HLA) system in humans, is located on the short arm of chromosome 6. MHC class I antigens (HLA-A, -B, and -C) are expressed on the surface of nearly all nucleated cells and mainly present endogenous peptide antigens to CD8 cytotoxic T cells. MHC class II antigens (HLA-DR, -DQ, and -DP) are mainly expressed on the surface of hematopoietic professional antigen presenting cells (B cells, monocytes, macrophages, and dendritic cells). However, many other hematopoietic-derived, epithelial, endothelial, and stromal cell populations can also express MHC class II, especially under inflammatory conditions (Zeiser and Blazar 2017a; Hill et al. 2021). MHC class II molecules present mainly exogenous peptide antigens to CD4 T cells. In contrast to MHC molecules, miHAs are polymorphic peptides bound to and presented by MHC molecules. They are generally ubiquitously expressed, but can differ in their tissue expression (Summers et al. 2020). This difference in expression among tissues may be one of the reasons why aGVHD predominantly involves the skin, liver, and gut. Some miHAs are also selectively expressed in the hematopoietic system and may be more potent targets of graft-versus-tumor rather than graft-versus-host responses (Summers et al. 2020). Minor histocompatibility antigen mismatches are most relevant to clinical aGVHD because the majority of clinical allogeneic transplants are MHC-matched.

The risk of acute GVHD is directly related to the degree of histocompatibility antigen mismatch (Zeiser and Blazar 2017a). For this reason, the optimal HSC donor is an MHC-matched related donor (MRD). Related donor grafts presumably have better outcomes in part due to less miHA mismatches. Unfortunately, aGVHD still occurs in 40% of patients who receive fully-matched grafts and immunosuppressive prophylaxis (Ferrara et al. 2009).

Most centers define an MHC-matched graft as one that is matched at the allelic level for HLA-A, -B, -C, and -DRB1 with minor clinical benefit for allelic matching at HLA-DQ, HLA-DP, and DR3/4/5 (Dehn et al. 2019). The minimal amount of MHC matching varies based on the HSC source. For bone marrow and peripheral blood-derived grafts, 8/8 matches are ideal, but 7/8-mismatched grafts can be used when better matched donors are unavailable (Dehn et al. 2019). However, aGVHD and mortality are increased with mismatched donors compared to matched donors, and the aGVHD prophylaxis for these donors is typically more immune suppressive (Jagasia et al. 2012; Flowers et al. 2011; Loiseau et al. 2007). Engraftment of umbilical cord blood HSCs is routinely achieved with greater than or equal to a 4/6 match (HLA-A, -B, -DR) using antigen-level matching for HLA-A and -B and allelic matching at HLA-DR, but mortality is lower when two or greater allelic mismatches are present within HLA-A, -B, -C, or -DR. (Dehn et al. 2019; Eapen et al. 2011; Eapen et al. 2017) Haploidentical donor grafts, as their name implies, can successfully engraft when the donor and recipient are half-matched. Acute GVHD prophylaxis for haploidentical donor transplantation typically employs post-transplant cyclophosphamide (PTCy) in addition to calcineurin-based regimens used for MRD transplantation (McCurdy and Luznik 2019).

The primary sources for donor stem cell grafts are the bone marrow and peripheral blood. Apheresis is used to harvest peripheral blood stem cell (PBSC) grafts following stem cell mobilization using hematopoietic growth factors such as granulocyte colony stimulating factor (G-CSF). Hematopoietic stem cells can also be obtained from umbilical cord blood (Ballen et al. 2013).

The T cell content of an HSC graft directly correlates with the risk of aGVHD. Peripheral blood-derived grafts carry the greatest T cell load followed by bone marrow and then umbilical cord blood grafts (Zeiser and Blazar 2017a; Flowers et al. 2011; Gooptu and Koreth 2020). Typically, HSC grafts are infused without altering their immune cell content. However, many approaches are being explored to reduce the T cell load of HSC grafts prior to infusion. These include positive selection of $CD34^+$ stem cells, depletion of αβ T cells, and depletion of naïve T cells, which are naïve to their cognate antigen and are more potent inducers of aGVHD relative to antigen-experienced memory T cells (Gooptu and Koreth 2020). One benefit of these approaches is that they often require less immunosuppressive aGVHD prophylaxis. However, because alloimmune T cell-mediate GVT and aGVHD are closely linked, relapse rates are often higher with T cell-depleted grafts (Gooptu and Koreth 2020). T cells are also critical for engraftment and immune recovery; therefore, T cell-depleted grafts often have higher rates of graft failure and infections (Gooptu and Koreth 2020).

Prior to administration of the HSC graft, recipients typically receive conditioning therapy to eradicate their malignancy and promote HSC engraftment. The intensity of conditioning regimens varies based on each patient's disease type, disease status, overall health and donor stem cell source (Zeiser and Blazar 2017a; Jagasia et al. 2012). Full intensity, myeloablative conditioning regimens are typically associated with a greater risk of aGVHD (Jagasia et al. 2012; Nakasone et al. 2015). This is thought to be due to greater tissue injury from these full intensity regimens. The

tissue injury causes the release of danger associated molecular patterns (DAMPs) and pathogen associated molecular patterns (PAMPs) that then activate antigen presenting cells resulting in the secretion of pro-inflammatory cytokines and the robust activation of allogeneic T cells (Zeiser and Blazar 2017a).

4 Chronic GVHD

Chronic GVHD (cGVHD) is a significant risk factor for nonrelapse mortality in patients two years or greater post allo-HSCT (Zeiser and Blazar 2017b). It is classically defined as occurring >100 days post-HSCT; however, it can occur earlier and present as an overlap syndrome with features of both acute and chronic GVHD. Chronic GVHD occurs in 30–70% of allo-HSCT recipients. It can arise de novo (i.e., in the absence of any prior aGVHD); however, it more commonly arises progressively (i.e., aGVHD transitions into cGVHD) or following a period of quiescent aGVHD (i.e., prior aGVHD resolves and then cGVHD develops) (Ferrara et al. 2009). Virtually every organ system can be affected by cGVHD, which resembles an "autoimmune syndrome" (Zeiser and Blazar 2017b; Saidu et al. 2020). Common manifestations include lichen planus-like skin lesions, sclerosis, myositis, fasciitis, vulvo-vaginitis, bronchiolitis obliterans (BO), sicca syndrome, and damage of the gastrointestinal tract and liver (Ferrara et al. 2009; Zeiser and Blazar 2017b; Saidu et al. 2020). Diagnosis, staging, and response grading of cGVHD are based on the National Institutes of Health Consensus Criteria (Lee et al. 2015; Jagasia et al. 2015). Risk factors include prior aGVHD, HLA-mismatched grafts, peripheral blood stem cell grafts relative to bone marrow grafts, older age of the recipient or donor, and transplantation of female grafts into male recipients (Flowers et al. 2011).

The immunobiology of cGVHD is complex and distinct from that of aGVHD. Briefly, it can be conceptualized in three phases: (1) inflammation causing tissue damage, (2) chronic inflammation leading to thymic injury as well as B and T cell dysregulation, and (3) tissue repair and often debilitating fibrosis (Hill et al. 2021; Zeiser and Blazar 2017b). A more detailed description of cGVHD immunobiology and management with immune suppression is outside the scope of this review. However, aGVHD is one of the greatest risk factors for cGVHD, and the immunosuppressive agents used to prevent and treat cGVHD often overlap with aGVHD (Zeiser and Blazar 2017b; Saidu et al. 2020; Grube et al. 2016). Therefore, we will point out those immunosuppressive agents used for both acute and chronic GVHD.

5 Immunobiology of aGVHD

The pathophysiology of aGVHD comprises a donor allogeneic T cell-dependent response to disparate histocompatibility antigens that results in the induction of pro-inflammatory cytokines and cellular effectors that damage target organs. Conceptually, it can be thought of as a destructive, unchecked immune response to foreign antigens. Acute GVHD pathogenesis consists of three phases. In phase I,

tissue injury from conditioning therapy causes inflammatory cytokine production and activation of APCs. In phase II, donor allogeneic CD4 and CD8 T cells recognize alloantigens, become activated, expand, and differentiate into effector T cells. In phase II, effector T cells and additional inflammatory mononuclear subsets traffic to aGVHD target organs and cause direct cell-mediated or indirect inflammatory cytokine-mediated tissue damage (Antin and Ferrara 1992). Similar to an immune response to a pathogen, the immunobiology of aGVHD consists of triggers, sensors, mediators, effectors, amplifiers, and modulators (Reddy 2012). While these frameworks are useful to conceptualize aGVHD pathophysiology, it is important to understand that aGVHD is a complicated systemic process with still many unknowns. Furthermore, a majority of aGVHD pathophysiology is based on murine studies. Therefore, it is worth noting that these studies are limited by differences in genetic heterogeneity, basic physiology, immune responses, microbiomes, environmental exposures, and HSCT procedures between laboratory mice and humans. Nonetheless, the rich understanding of aGVHD pathophysiology in murine models is the foundation of many immunosuppressive therapies for aGVHD prevention and treatment.

5.1 Tissue Injury and Inflammation from Pre-transplant Conditioning (aGVHD Triggers and Sensors)

Tissue damage from conditioning is the earliest trigger of aGVHD. Damaged tissues release endogenous DAMPs, including uric acid and adenosine triphosphate (ATP) (Zeiser and Blazar 2017a; Wilhelm et al. 2010; Jankovic et al. 2013). In the gut, damaged epithelium allows for the translocation of exogenous PAMPs, such as lipopolysaccharide (bacterial component), CpG oligodeoxynucleotides (viral DNA), and α-mannan (fungal component) (Zeiser and Blazar 2017a). Alarmin molecules (IL-1α, IL-33, and HMGB1) are also released. DAMPs, PAMPs, and alarmins are then recognized by pattern recognition receptors (PRRs) (e.g., NOD-like receptors and Toll-like receptors) and alarmin receptors in host tissues (Hill et al. 2021). Ligand-bound PRRs and alarmin receptors initiate signaling pathways (e.g., NF-κB) that activate cytokine (e.g., TNFα, IL-1β, IL-6, IL-33, IL-12, IL-23, type I IFNs) and chemokine (e.g., CCL5) production (Zeiser and Blazar 2017a; Hill et al. 2021; Hill and Koyama 2020). These inflammatory cytokines and chemokines recruit myeloid cells including monocytes and neutrophils, which cause further tissue damage, particularly in the GI tract, through their production of reactive oxygen species (Zeiser and Blazar 2017a; Hill et al. 2021; Hill and Koyama 2020).

APCs are the main sensors of aGVHD. The inflammatory environment created by the conditioning regimen activates host APCs (e.g., dendritic cells and macrophages) (Zeiser and Blazar 2017a; Hill et al. 2021). Activated APCs increase allo-antigen presentation, upregulate co-stimulatory molecules, and secrete inflammatory cytokines (Zeiser and Blazar 2017a; Hill et al. 2021). In this way, activated APCs provide the primary, secondary, and tertiary signals needed for the activation of

donor allogeneic T cells, which are the primary mediators of aGVHD. Host APCs, particularly dendritic cells (DCs), are thought to be the most potent activators of allo-T cells early post-transplant. However, donor APCs in general and donor $CD103^+$ DCs specifically migrate to lymphoid tissues where they also activate allo-reactive T cells that potentiate aGVHD (Hill et al. 2021; Koyama et al. 2015). Allogeneic antigens are also presented by non-hematopoietic host tissues (Koyama et al. 2011; Koyama et al. 2019; Toubai et al. 2012). For example, damage from conditioning induces IL-12 secretion from intestinal macrophages that then drives the production of IFN-γ from intestinal lymphocytes. IFN-γ then enhances MHC-II expression on intestinal epithelial cells thereby promoting CD4 T cell-mediated aGVHD (Koyama et al. 2019).

5.2 Stimulation, Differentiation, and Proliferation of Effector T Cells (aGVHD Mediators)

Donor allo-reactive T cells are the primary mediators of aGVHD. Upon infusion, they enter a lymphopenic, inflamed host, which promotes their profound proliferation (Hill et al. 2021). In murine models, naïve ($CD62L^+$ $CD45RA^+$ $CCR7^+$) T cells (i.e., antigen-inexperienced) are far more likely to cause aGVHD than memory T-cells (Hill et al. 2021; Chen et al. 2007). However, human recipients of naïve T cell-depleted grafts still develop aGVHD (Gooptu and Koreth 2020; Bleakley et al. 2015). Proliferating naïve T cells then traffic to lymph nodes where they become activated by disparate histocompatibility antigens on APCs. APCs also provide important secondary activation signals to these T cells through co-stimulatory molecules. Co-stimulatory pathways such as CD28, ICOS, OX40, and 4-1BB lower T cell activation thresholds, augment cytokine production, inhibit apoptosis, and support effector T cell metabolism (Zeiser and Blazar 2017a). Similarly, the Notch ligand DLL4 expressed on non-hematopoietic stromal cells also promotes allogeneic T cell-driven aGVHD (Hill and Koyama 2020; Chung et al. 2017).

Signal transduction downstream of the T cell receptor and co-stimulatory receptors starts with receptor-proximal phosphorylation of signaling molecules (Gaud et al. 2018; Huse 2009). This then promotes the activation of phospholipase C which hydrolyzes phosphatidylinositol bisphosphate (PIP_2) to yield diacylglycerol (DAG) and inositol trisphosphate (IP_3). DAG recruits a number of downstream signaling molecules including protein kinase C-θ (PKCθ) that results in the activation of the mitogen-activated protein kinase (MAPK) cascade and culminates in the activation of the transcription factor AP-1. PKCθ also induces a signaling pathway leading to the activation of the transcription factor NF-κB. Meanwhile, IP_3 causes calcium channels to open thereby raising the cytoplasmic calcium concentration. This promotes the activation of the protein phosphatase calcineurin, which dephosphorylates the transcription factor nuclear factor of activated T cells (NFAT). The end result of TCR signal transduction is the activation of the transcription factors NFAT, AP-1, and NF-κB that induce the expression of a number of

genes that promote the activation and proliferation of T cells including IL-2 (Gaud et al. 2018; Huse 2009).

Effector CD4 and CD8 T cells differentiate into helper (Th) and cytotoxic (Tc) subsets characterized by the cytokines they produce and the expression of subset-specific transcription factors (Hill et al. 2021; Fu et al. 2014). The inflammatory cytokine milieu present post-HSCT generally polarizes CD4 helper and CD8 cytotoxic T cells toward the inflammatory Th1/Th17 and Tc1/Tc17 subsets, respectively (Hill et al. 2021; Fu et al. 2014). Th1/Tc1 polarization is promoted by high levels of IL-12 and IFN-γ, and Th17/Tc17 polarization is promoted by high levels of IL-6 in combination with TGFβ. IL-6 also inhibits the induction of Tregs. In contrast to IL-12 and IFNγ, IL-4 levels, which support Th2/Tc differentiation, are generally minimally elevated post allogeneic HSCT. Th1/Tc1 are characterized by the production of the inflammatory cytokines IL-2, IFN-γ and TNF-α whereas Th17/Tc17 produce IL-17 and IL-21 (Hill et al. 2021; Fu et al. 2014). Th1/Tc17 and Tc1/Tc17 cells promote aGVHD. By contrast, Th2/Tc2 (secrete IL-4, IL-5, and IL-13) and Tregs (secrete IL-10 and TGFβ) ameliorate aGVHD (Hill et al. 2021; Fu et al. 2014). However, exceptions to these generalizations exist at least in part due to contextual differences among models. For example, IFN-γ is a characteristic cytokine of Th1 cells, and it is cytotoxic to intestinal epithelial cells (Takashima et al. 2019). Despite this, its absence in donor T cells is protective when mice are conditioned with low-dose irradiation and detrimental when conditioned with high-dose irradiation (Welniak et al. 2000). This discrepancy was shown to be due in part to IFN-γ's ability to protect against Th2-mediated lung damage (Hill et al. 2021; Fu et al. 2014). Nevertheless, donor T cells deficient for the Th1-specific transcription factor, T-bet, caused less severe aGVHD (Fu et al. 2015). In addition to model-dependent effects of T cell differentiation on aGVHD, the polarization of helper T cell subsets is reciprocally regulated. Disrupting this regulation in model systems skews helper T cell polarization, cytokine production, and T cell migration such that different organs are targeted depending on which helper T cell differentiation pathway is blocked (Yi et al. 2009).

Helper T cell subsets differentially express chemokine receptors that govern their trafficking to target tissues (Fu et al. 2014). Th1 cells express CCR5 and CXCR3, which aids their trafficking to the gut and liver, respectively (Fu et al. 2014). Th17 cells express CCR6 promoting trafficking to the skin, and Th2 cells express CCR4 allowing them to traffic to the lungs (Fu et al. 2014). This differential expression of chemokine receptors on inflammatory T cell subsets may contribute to the gut, liver, and skin being the primary aGVHD target organs. As a further example of how T cell trafficking influences aGVHD, colon-derived donor DCs migrate to mesenteric lymph nodes where they active donor T cells and imprint them with gut-homing expression of α4β7 integrin (Koyama et al. 2015). This leads to the migration of allogeneic T cells into the GI tract where they cause fulminant disease (Koyama et al. 2015).

5.3 Tissue Damage by Effectors and Inflammatory Cytokines (aGVHD Effectors and Amplifiers)

The effector phase leading to GVHD target organ damage is mediated by inflammatory monocytes, cytolytic cellular effectors (e.g., CD8 and CD4 T cells), inflammatory cytotoxic cytokines (e.g., IL-1β, TNFα, IFN-γ), and reactive oxygen species (ROS) (Zeiser and Blazar 2017a; Hill et al. 2021). GVHD organ damage caused by these effector mechanisms is further amplified by a vicious cycle of tissue damage, inflammation, recruitment of cellular effectors and secretion of cytotoxic cytokines (Zeiser and Blazar 2017a; Hill et al. 2021).

CD4 and CD8 T cells are the main cellular effectors of aGVHD. They are typically donor in origin, but recent evidence suggests that recipient tissue resident memory T cells may also cause tissue damage (Divito et al. 2020; Strobl et al. 2020). T cells typically kill target cells via contact-dependent mechanisms including activation of perforin-granzyme, Fas–FasL (CD95-CD95L), or TNFR-TNF-related apoptosis-inducing ligand (TRAIL) pathways (Du and Cao 2018; Shlomchik 2007). Perforin and granzyme are stored in the cytotoxic granules of cytotoxic T lymphocytes (CTLs) and are secreted upon recognition of target cells. Perforin forms pores in target cells through which granzyme passes. Granzyme then induces apoptotic death in target cells by releasing mitochondrial cytochrome C. Fas clustering on the surface of target cells is induced by binding to FasL on T cells, resulting in the formation of a death-inducing signal complex and the triggering of apoptosis on target cells (Du and Cao 2018). Other CTL killing mechanisms involve TNF death ligand receptor–triggered apoptosis by activation of the TNF/TNFR, TRAIL, TNF-related weak inducer of apoptosis (TWEAK), and lymphotoxin ß (LTß)/LIGHT pathway (Reddy 2012).

Inflammatory pathways do not require cell–cell contact to kill target cells. Instead, target cell damage is caused by cytotoxic cytokines (TNFα and IFNγ) and ROS released by allogeneic T cells and inflammatory monocytes, respectively (Zeiser and Blazar 2017a; Schwab et al. 2014). It is important to note that both the cell-mediated and inflammatory cytotoxic cytokine-mediated effector pathways are important for GVL effects as well as negative feedback on inflammatory components driving aGVHD (Hill et al. 2021; Du and Cao 2018). Therefore, the utility of therapeutically targeting aGVHD effector mechanisms is uncertain.

5.4 Tissue Repair and Anti-inflammatory Mechanisms (aGVHD Modulators)

There are many immune cell-related and non-immune cell-related mechanisms that modulate aGVHD pathophysiology and contribute to tissue repair. For instance, activated allogeneic T cells express not only co-stimulatory receptors but also co-inhibitory receptors that attenuate allo-T cell responses and suppress aGVHD such as CTLA-4, PD-1, BTLA, LIGHT, LAG3, TIGIT and VISTA (Zeiser and Blazar 2017a; Hill et al. 2021). In addition, many cytokines secreted by activated T

cells (e.g., IFNy, IL-12, IL-22, IL-10, TGFβ, and IL-2) have both pro- and anti-aGVHD affects depending on the context and model system (Zeiser and Blazar 2017a; Hill et al. 2021; Hill and Koyama 2020). APCs also have dual effects on aGVHD that vary by context and the subset examined. As an example, both host and donor DCs promote aGVHD whereas host $CD8^+$ DCs and donor pre-plasmacytoid DCs inhibit aGVHD (Yu et al. 2019). Furthermore, the ability of dendritic cells to promote inflammatory or tolerogenic immune responses can be modified. For instance, co-transplantation of ex vivo-derived regulatory DCs inhibits aGVHD in murine models (Sato et al. 2003). One promising way of promoting a tolerogenic DC phenotype in vivo is to administer histone deacetylase inhibitors (HDACi), which improve aGVHD in both pre-clinical and clinical studies (Li et al. 2020; Choi and Reddy 2011).

Similar to DCs, macrophages are an APC that also regulates aGVHD in complex ways (Hong et al. 2020). Blocking their recruitment to target organs inhibits aGVHD, and the anti-aGVHD activity of corticosteroids appears to be in part due to the inhibition of macrophages (Nishiwaki et al. 2014; Cheng et al. 2015). However, other studies have shown that host macrophages attenuate aGVHD in murine models (Nieves et al. 2017; Hashimoto et al. 2011). The influence of inflammatory M1 macrophages relative to anti-inflammatory M2 macrophages on aGVHD is also complex. One study found an elevated M2 macrophage gene signature in colon biopsies from steroid-refractory aGVHD patients (Holtan et al. 2019). By contrast, G-CSF-mobilized HSCT grafts with higher levels of M2 macrophages were associated with less subsequent aGVHD (Wen et al. 2019).

A subset of monocytic and granulocytic myeloid cells, termed myeloid derived suppressor cells (MDSC), are highly immune suppressive (Zeiser and Blazar 2017a; Voermans and Hazenberg 2020). Adoptively transferred MDSCs promoted tolerogenic Th2 and Treg responses thereby suppressing murine aGVHD (Voermans and Hazenberg 2020; Ghansah et al. 2004; Vendramin et al. 2014; Fan et al. 2017; Wang et al. 2019; Highfill et al. 2010; Zhang et al. 2019). However, MDSCs can lose their suppressor function by inflammasome activation when in pro-inflammatory environments (Koehn et al. 2015; Koehn et al. 2019). Due to this, repeat MDSC infusion is often required to control aGVHD in murine models.

Mesenchymal stromal cells (MSC) may also be useful for the treatment of aGVHD. MSCs are typically derived from bone marrow, umbilical cord blood, or adipose tissue. They express CD73, CD90, and CD105 and lack expression of CD34, CD45, CD14, CD11b, CD79a, CD19, and HLA-DR. (Voermans and Hazenberg 2020; Cheung et al. 2020) They are further defined by their ability to adhere to tissue culture plates and differentiate into osteoblasts, adipocytes, and chondroblasts. MSCs express little if any MHC-I or MHC-II allowing them to be administered across HLA barriers. These cells possess immunosuppressive capabilities in inflammatory environments via a variety of mechanisms including apoptotic death of the MSCs by host immune cells. The apoptotic MSCs are then phagocytosed which promotes the secretion of anti-inflammatory mediators that regulate both innate and adaptive immune cells. Due to their limited survival in the host, multiple infusions are required (Voermans and Hazenberg 2020; Cheung

et al. 2020). A number of small heterogeneous studies showed variable responses of steroid-refractory aGVHD (SR-aGVHD) to MSC therapy (Voermans and Hazenberg 2020; Cheung et al. 2020). One multicenter, randomized controlled trial did not meet its primary endpoint of improved durable complete remission (Kebriaei et al. 2020). However, overall responses were significantly higher in pediatric and high-risk patients. MSC efficacy in pediatric SR-aGVHD was also shown in a prospective, single-arm, phase 3 study (Kurtzberg et al. 2020). Importantly, MSCs are safe and well tolerated (Voermans and Hazenberg 2020; Cheung et al. 2020). Despite clinical trials showing inconsistent results, they are increasingly being used for aGVHD especially in the steroid-refractory setting.

Regulatory T cells are classically defined as $CD4^+$ $FOXP3^+$ $CD25^+$ cells with immunosuppressive capacity. $CD8^+$ and $FOXP3^-$ regulatory T cell subsets have also been described, but the role of $CD4^+$ $FOXP3^+$ $CD25^+$ Tregs is far more established in aGVHD (Hill et al. 2021). $CD4^+$ $FOXP3^+$ $CD25^+$ Tregs arise directly following thymic maturation or are induced in the periphery from CD4 T cells (Mancusi et al. 2019). Acute GVHD is associated with deficient Treg reconstitution and reduced Treg function in pre-clinical and clinical studies (Mancusi et al. 2019; Elias and Rudensky 2019). Enhancing or adoptively transferring donor Tregs in pre-clinical models increases the ability of Tregs to suppress conventional allogeneic T cells and prevent or mitigate aGVHD (Mancusi et al. 2019; Elias and Rudensky 2019; Taylor et al. 2002; Nguyen et al. 2007). In early-phase clinical trials, adoptive transfer of Tregs appears safe and effective for aGVHD prevention without causing greater leukemia relapse (Meyer et al. 2019; Di Ianni et al. 2011; Martelli et al. 2014; Brunstein et al. 2016). The ability of Tregs to treat clinic aGVHD remains to be determined (Trzonkowski et al. 2009). Major limitations of adoptive Treg therapy include that their ex vivo expansion is challenging and that they often convert to non-regulatory conventional T cells in inflammatory environments (Hill et al. 2021; Mancusi et al. 2019; Elias and Rudensky 2019). Therefore, another approach has been to enhance Treg recovery and activity in vivo by taking advantage of their increased IL-2 receptor expression and relative heightened dependence on IL-2 for survival compared to conventional T cells. Consistent with this, low-dose IL-2 therapy preferentially expanded Tregs relative to conventional T cells and mitigated chronic GVHD in a phase 1 clinical trial (Koreth et al. 2011; Matsuoka et al. 2013). Calcineurin inhibitors (CNIs), which are commonly used for aGVHD prophylaxis, inhibit IL-2 production and may hinder Treg recovery post-HSCT (Zeiser et al. 2006). However, the mTOR inhibitor, rapamycin, has less of an effect on IL-2 production, and when combined with low-dose IL-2, it expanded Tregs in vivo (Zeiser et al. 2006; Whitehouse et al. 2017; Furlan et al. 2020).

Alpha-1-antitrypsin (AAT) is a serine protease inhibitor produced by the liver and is lost through the GI tract especially with GI aGVHD (Rodriguez-Otero et al. 2012). In murine models, AAT administration was effective at preventing and treating aGVHD (Tawara et al. 2012; Marcondes et al. 2014). The anti-aGVHD mechanism of AAT is not clear, but may involve promoting Treg recovery and altering inflammatory cytokine production (Tawara et al. 2012; Marcondes et al. 2014; Magenau

et al. 2018). A phase 2 clinical trial showed promising responses in steroid-refractory acute GVHD (Magenau et al. 2018).

B cells are lymphoid cells best known for their production of antibodies and their ability to present antigens. The role of B cells in aGVHD is nuanced. B cell depletion prior to HSCT in mice and humans inhibited aGVHD (Kebriaei et al. 2020; Shimabukuro-Vornhagen et al. 2009; Schultz et al. 1995; Kamble et al. 2006; Ratanatharathorn et al. 2009; Khouri et al. 2008; Shimoni et al. 2003; Christopeit et al. 2009). Human HSCT grafts with high numbers of B lymphocytes correlated with an increased incidence of aGVHD (Iori et al. 2008). In contrast to these studies suggesting that B cells aggravate aGVHD, studies in mice also showed that B cells inhibit aGVHD by producing the anti-inflammatory cytokine IL-10 (Weber et al. 2014). Co-transfer of regulatory B cells also attenuated murine aGVHD (Hill et al. 2021; Hu et al. 2017). In humans, grafts with a high content of B cell progenitors are associated with less aGVHD (Michonneau et al. 2009). Altogether, these studies suggest that B cells likely modulate aGVHD in a context and subset-dependent manner.

NK cells are innate lymphoid cells with important antitumor and antimicrobial properties. They are the first donor lymphoid cell to recover post-HSCT (Simonetta et al. 2017). Their effect on aGVHD is also variable and likely depends on incompletely understood contextual factors. Early studies in mice and humans suggested that NK cells promoted aGVHD (Simonetta et al. 2017; Acevedo et al. 1991; Roy et al. 1993; Guillén et al. 1986). However, subsequent studies suggested that NK cells regulated alloimmune T cells via direct cytotoxic mechanisms resulting in less aGVHD (Simonetta et al. 2017; Murphy et al. 1992; Ruggeri et al. 2002; Olson et al. 2010). By contrast, recent studies also suggest activated NK cells administered at later time points post-HSCT may augment aGVHD via inflammatory cytokine-mediated indirect activation of alloimmune T cells (Simonetta et al. 2017; Xun et al. 1995; Xun et al. 1993; Cooley et al. 2005; Shah et al. 2015). Nonetheless, most clinical studies of adoptively transferred NK cells did not increase the incidence of aGVHD (Simonetta et al. 2017; Passweg et al. 2004; Choi et al. 2014a; Jaiswal et al. 2017).

Invariant natural killer cells (iNKT) are $CD3^+$, $CD4^+$, or $CD4^-$ cells that express NK cell markers and an invariant αβ TCR. Invariant NKT cells respond to lipid molecules presented by the non-polymorphic MHC-I-like CD1d molecule (Voermans and Hazenberg 2020). When activated, these cells promote tolerance by secreting IL-4 and IL-13 (Voermans and Hazenberg 2020; Andrlová et al. 2020). Human grafts with high iNKT cells numbers are associated with a lower incidence of aGVHD (Chaidos et al. 2012). In mice, iNKT cells protected against aGVHD (Voermans and Hazenberg 2020; Andrlová et al. 2020; Schneidawind et al. 2014; Schneidawind et al. 2015). In humans, the iNKT agonist RGI-2001 decreased the incidence of aGVHD (Chen et al. 2017a). These data overall suggest that targeting iNKT cells may be a promising approach for preventing aGVHD.

Mucosal-associated invariant T (MAIT) cells express a semi-variant TCR that recognizes microbial vitamin B biosynthesis intermediates presented by the monomorphic MHC-I-related molecule, MR1 (Andrlová et al. 2020). Mouse studies show that recipient MAIT cells reduce GI aGVHD by promoting intestinal barrier function in an IL-17-dependent manner (Varelias et al. 2018). The association of MAIT cell reconstitution and clinical aGVHD is variable and requires further study (Voermans and Hazenberg 2020; Bhattacharyya et al. 2018; Ben Youssef et al. 2018; Kawaguchi et al. 2018).

Gamma-delta (γ/δ)T cells are unconventional T cells activated by phosphoantigens (Andrlová et al. 2020). Their role in aGVHD is uncertain. Murine models demonstrated that both host and recipient γ/δ T cells exacerbated aGVHD (Blazar et al. 1996; Maeda et al. 2005). However, the clinical evidence for human γ/δ T cells exacerbating aGVHD is variable (Andrlová et al. 2020). Consistent with a minimal contribution of human γ/δ T cells to aGVHD, α/β T cell-depleted grafts, which are enriched in γ/δ T cells, are well tolerated (Locatelli et al. 2017; de Witte et al. 2021).

Innate lymphoid cells (ILC) lack rearranged antigen receptors and share a common progenitor with NK cells. ILCs are classified into ILC1, ILC2, and ILC3 subsets that possess cytokine repertoires similar to that of Th1, Th2, and Th17 cells (Voermans and Hazenberg 2020; Shao et al. 2019). Secretion of IL-22 by recipient ILC3 cells protected intestinal stem cells from allogeneic T cell-mediated damage and ameliorated aGVHD in mice (Hanash et al. 2012). Transfer of donor ILC2 cells treated established murine aGVHD by activating anti-inflammatory MDSCs in an IL-13-dependent manner (Bruce et al. 2017). Delayed ILC reconstitution in humans has also been associated with a higher risk for aGVHD (Munneke et al. 2014). A clear role for ILC1 cells in the pathogenesis of aGVHD has not yet been determined.

The gut microbiome is critical for the homeostasis of the digestive and immune systems. Growing evidence indicates that dysregulation of the gut microbiome following allogeneic HSCT worsens aGVHD (Zeiser and Blazar 2017a; Hill et al. 2021; Rafei and Jenq 2020). Microbiome dysbiosis occurs following allo-HSCT due to broad-spectrum antibiotic use, conditioning therapy, and changes in host nutrition secondary to mucositis, nausea, and vomiting from the conditioning therapy (Rafei and Jenq 2020). This dysbiosis can skew microbial populations and their metabolites. For instance, the short chain fatty acid microbial metabolite butyrate is reduced in murine models of aGVHD (Mathewson et al. 2016). Supplementation with butyrate or butyrate-producing bacteria ameliorated GI aGVHD by protecting intestinal epithelial cells from allo-T cell-mediated damage (Mathewson et al. 2016). Indole metabolites derived from microbial metabolism of tryptophan also protected mice from GI aGVHD via a type I IFN-dependent mechanism (Swimm et al. 2018). In addition to microbial metabolites, prebiotics such as lactose have also been shown to promote aGVHD by driving the outgrowth of aGVHD-associated *Enterococcus (*Stein-Thoeringer et al. 2019*)*. Host factors secreted into the intestinal lumen, such as defensins and regenerating proteins, also mitigate acute GI GVHD by protecting the intestinal epithelium from bacterial translocation and decreasing crypt apoptosis

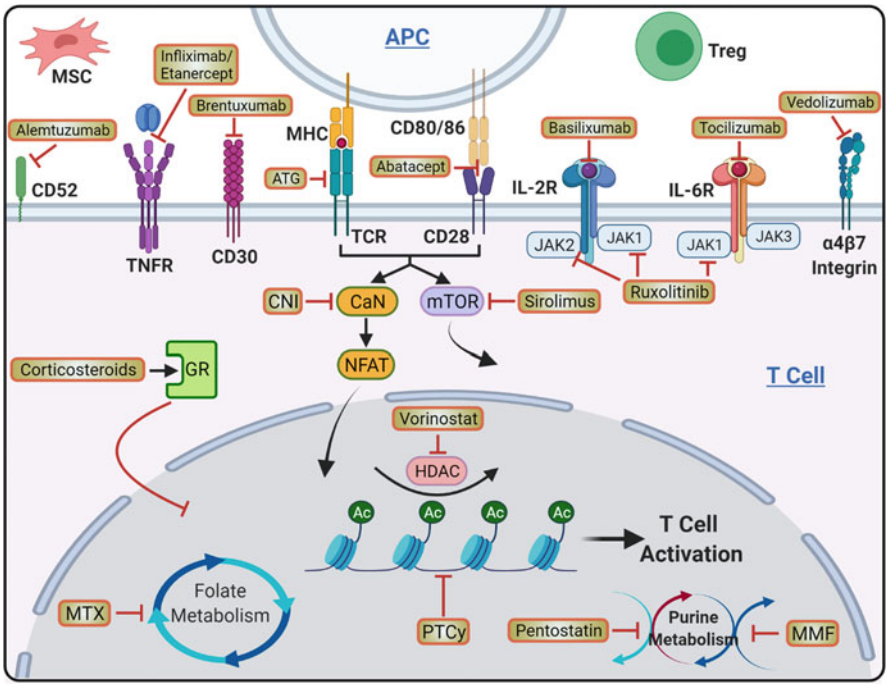

Fig. 1 Immune suppressive therapies for aGVHD prevention and treatment. *Ac* Acetylated, *APC* Antigen presenting cell, *ATG* Anti-thymocyte globulin, *CaN* Calcineurin, *CNI* Calcineurin inhibitor, *GR* Glucocorticoid receptor, *HDAC* Histone deacetylase, *JAK* Janus kinase, *MHC* Major histocompatibility complex, *MMF* Mycophenolate mofetil, *MSC* Mesenchymal stromal cell, *mTOR* Mammalian target of rapamycin, *MTX* Methotrexate, *NFAT* Nuclear factor of activated T cells, *PTCy* Post-transplantation cyclophosphamide, *Treg* Regulatory helper T cell. This image was made using BioRender

(Zeiser and Blazar 2017a; Zhao et al. 2018). The Wnt agonist, R-spondin-1, augments this process by protecting intestinal stem cells from aGVHD and expanding Paneth cells, which are then able to secrete more antimicrobial defensins (Hayase et al. 2017; Takashima et al. 2011).

In summary, the immunobiology of aGVHD is complex and involves essentially all aspects of the immune system. Allo-reactive T cells are central to aGVHD pathophysiology and have been the main target of both treatment and prophylactic immune suppressive agents for aGVHD over the last 30 years. With greater mechanistic understanding of aGVHD immunobiology, additional therapeutic agents have been and continue to be developed. In the following sections, the immune suppressive strategies used to prevent and treat aGVHD (Fig. 1) and additional immune dysregulation conditions associated with hematopoietic stem cell transplantation are described (Table 1).

Table 1 Immune suppressive therapies for various hematopoietic stem cell transplantation indications

Drug	Mechanism of action	Primary indication	Notable adverse effects
Tacrolimus/ cyclosporine (CSA)	Calcineurin inhibition	GVHD prophylaxis	Hypomagnesemia (tacrolimus), renal dysfunction, hypertension, PRES, TMA, gingival hyperplasia (CSA), hirsutism (CSA), viral infections
Mycophenolate mofetil	Inhibiting the enzyme inosine monophosphate dehydrogenase	GVHD prophylaxis	JC virus-associated progressive multifocal leukoencephalopathy, viral infections, hypertension, peripheral edema, hyperglycemia, cytopenias, nephrotoxicity, liver injury
Methotrexate	Dihydrofolate reductase suppression	GVHD prophylaxis	Nephrotoxicity, cytopenias, gastrointestinal issues, oral mucositis: leucovorin rescue imperative
Sirolimus	Mammalian target of rapamycin (mTOR) inhibition	GVHD prophylaxis	Hypertriglyceridemia, impaired wound healing, renal impairment, oral ulcers, gastrointestinal complaints, increased risk of infections
Anti-thymocyte globulin	T lymphocyte destruction	GVHD prophylaxis	Serum sickness, infusion reaction, viral reactivation
Cyclophosphamide	Alkylating agent resulting in T cell modifications	GVHD prophylaxis	Cardiotoxicity, myelosuppression, nephrotoxicity, hemorrhagic cystitis, nausea/vomiting
Vorinostat	Histone deacetylase inhibitor	GVHD prophylaxis	Hepatic injury, electrolyte abnormalities, risk for bacterial infections, cardiac arrhythmias (QTc prolongation), mucositis
Abatacept	CTLA-4 analog	GVHD prophylaxis	Viral infections, hypersensitivity reaction, headaches, nausea
Maraviroc	CCR5 blockade	GVHD prophylaxis	Dizziness, hepatotoxicity, risk of infections, hypersensitivity, skin rash, vomiting, fever
Sitagliptin	Inhibition of dipeptidyl peptidase 4 (DPP-4)	GVHD prophylaxis	Hypoglycemia, rash, acute pancreatitis, liver toxicity, nephrotoxicity

(continued)

Table 1 (continued)

Drug	Mechanism of action	Primary indication	Notable adverse effects
Prednisone/ methylprednisolone, budesonide/ beclomethasone	Corticosteroids (systemic/enteral)	GVHD treatment	Opportunistic infections including pneumocystis, hyperglycemia, hypertension, hepatic cirrhosis, avascular necrosis
Ruxolitinib	JAK1/2 inhibition	GVHD treatment	Cytopenias (thrombocytopenia, anemia), hepatic toxicity, increased infectious risk, elevated serum cholesterol, hypertriglyceridemia
Infliximab, etanercept	Tumor necrosis factor inhibitors	GVHD treatment, treatment of IPS	Infusion reactions (acute and delayed), opportunistic infections, hepatic toxicity, anemia, abdominal pain, rash
Alemtuzumab	anti-CD52	GVHD treatment	Prolonged significant lymphopenia, high infection risk, infusion reaction, thyroid disease, cytopenias, autoimmune hepatitis, skin rash, fever
Pentostatin	Purine analog	GVHD treatment	Infections, lymphopenia, pulmonary dysfunction, gastrointestinal complaints, central nervous system toxicity, rash, hepatitis, fatigue, fever
Basiliximab, daclizumab	Interleukin-2 Receptor (CD25-alpha) antibodies	GVHD prophylaxis/ treatment	Viral infections, hypertension, hyperglycemia, electrolyte abnormalities, hepatic toxicity, rash
Brentuximab	anti-CD30	GVHD treatment	Acute pancreatitis, neuropathy, hyperglycemia, infusion-related reactions, neutropenia, hepatotoxicity
Tocilizumab	Interleukin-6 receptor monoclonal antibody	GVHD treatment	Respiratory tract and cutaneous infections, neutropenia, mycobacterium reactivation, increased serum cholesterol, transaminitis, infusion-related reactions, hypertension

(continued)

Table 1 (continued)

Drug	Mechanism of action	Primary indication	Notable adverse effects
Vedolizumab	Inhibition of mucosal addressin cell adhesion molecule-1 (MAdCAM-1) and alpha4beta7 integrin interaction	Gastrointestinal GVHD treatment	*C. difficile* disease, infusion-related reactions, headache, arthralgias
Rituximab	anti-CD20	Post-transplant immune-mediated cytopenias, EBV viremia, GVHD prevention	Hypogammaglobulinemia, B cell lymphopenia, infusion-related hypersensitivity, fever, hepatitis B reactivation
Bortezomib	Proteasome inhibitor	Post-transplant immune-mediated cytopenias	Peripheral neuropathy, posterior reversible leukoencephalopathy syndrome, hepatotoxicity, cardiac dysfunction, herpes zoster reactivation, gastrointestinal issues
Eculizumab	Inactivation of terminal complement component CD5	Treatment of TMA	Significant risk for meningococcal disease and encapsulated organisms (antimicrobial prophylaxis required), hypertension/tachycardia, headache, hypokalemia, rash, diarrhea/nausea/vomiting, anemia/leukopenia, fever, renal insufficiency

GVHD Graft-versus-host disease, *PRES* Posterior reversible encephalopathy syndrome, *TMA* Thrombotic microangiopathy, *CTLA-4* Cytotoxic T-lymphocyte-associated antigen-4, *CCR5* C-C Chemokine receptor type 5, *IPS* Idiopathic pneumonia syndrome

6 GVHD Prophylaxis

6.1 Calcineurin Inhibitors

Primary GVHD prophylaxis revolves around the usage of CNIs, most prominently tacrolimus and cyclosporine (Choi et al. 2010; Gatza et al. 2020). Calcineurin inhibitors primarily prevent GVHD by blocking allogeneic T cell proliferation and IL-2 production (Chinen and Shearer 2010; Heidt et al. 2010; Choi and Reddy 2014). They are associated with electrolyte abnormalities (hypomagnesemia notably with tacrolimus), nephrotoxicity, and hypertension. Close therapeutic drug

monitoring to ensure target trough levels can lessen many of these adverse risks. Gingival hyperplasia and hirsutism may additionally be seen with cyclosporine usage. Of note, tacrolimus and cyclosporine appear to also be associated with the serious post-transplant conditions of thrombotic microangiopathy (TMA) and posterior reversible encephalopathy syndrome (PRES). Given an increased risk of viral infections with their usage, Epstein-Barr virus-associated post-transplant lymphoproliferative disease may be observed with CNIs. Despite the mentioned risks and necessity for close monitoring, CNIs are overall well tolerated and have been a cornerstone of aGVHD prophylaxis for decades.

6.2 Mycophenolate Mofetil

Concurrent usage of CNIs and mycophenolate mofetil (MMF) in the prevention of GVHD continues to be explored. Most studies to date have evaluated MMF usage in non-myeloablative and reduced intensity conditioning regimens (Choi and Reddy 2014; Ruutu et al. 2014). By inhibiting the enzyme inosine monophosphate dehydrogenase (IMPDH), which lymphocytes particularly rely on for purine synthesis, mycophenolate acts by reducing lymphocyte proliferation (Gatza et al. 2020; Cuny et al. 2017). Infectious risks with MMF include JC virus-associated progressive multifocal leukoencephalopathy (PML), disseminated CMV or EBV, and reactivation of hepatitis B or C. Adverse drug reactions include peripheral edema, hypertension, hyperglycemia, nausea/vomiting, drug-related cytopenias, nephrotoxicity, and hepatic injury.

6.3 Methotrexate

Low-dose intravenous methotrexate plus a CNI has also shown efficacy in the prevention of GVHD. Methotrexate impedes T cell activation by inhibiting dihydrofolate reductase resulting in impairment of lymphocyte DNA synthesis and repair. Dosing ranges from 10–15 mg/m^2 on days +1, +3, +6, and + 11 following allogeneic transplantation (Choi et al. 2010; Nash et al. 1996). Leucovorin rescue is additionally administered in an effort to reduce toxicity to the kidneys, gastrointestinal tract, and oral mucosa. However, such adverse effects are much less commonly seen than with anti-neoplastic high-dose methotrexate regimens. Leucovorin prevents these toxicities by displacing methotrexate from binding sites allowing cells to once again proceed with RNA and DNA synthesis.

6.4 Sirolimus

Sirolimus acts via suppression of the mammalian target of rapamycin (mTOR) pathway leading to reduced IL-2 production and resultant blockage of T cell growth and proliferation. The agent has typically been used in combination with tacrolimus

and methotrexate for the prevention of GVHD (Choi and Reddy 2014). Initial studies showed promise with the therapy, but later trials appeared to reveal a possible increased risk of veno-occlusive disease (VOD) and thrombotic microangiopathy (TMA) in those receiving sirolimus (Pulsipher et al. 2014). Further studies are needed and are undergoing to fully understand the potential benefit of the agent in prevention of GVHD. Additional toxicities include hypertriglyceridemia, impaired wound healing, renal impairment, oral ulcers, and gastrointestinal complaints, including loose stools.

6.5 Anti-Thymocyte Globulin

Polyclonal immunoglobulins targeting human T lymphocytes, e.g., anti-thymocyte globulin (ATG) therapy, may be beneficial in the prevention of acute and chronic GVHD, but a strong survival benefit has not been observed (Arai et al. 2017). When administered prior to donor cell infusion, they assist in reducing graft rejection, while the GVHD-related benefits are seen with delivery post-donor cell infusion. Adverse events to be aware of include risk for anaphylaxis, serum sickness with fever, and viral reactivation, including EBV and CMV.

6.6 Cyclophosphamide

Post-transplant cyclophosphamide (PTCy) on days +3 and +4 has been found to reduce the incidence of acute and chronic GVHD through possible reduction of allo-reactive T cells with additional effects on regulatory T cells (Gatza et al. 2020; Choi and Reddy 2014; Wachsmuth et al. 2019; Kanakry et al. 2013). This alkylating agent is now widely used and considered well tolerated even in the setting of additional calcineurin inhibition or MMF administration. The risk of hemorrhagic cystitis is reduced with aggressive intravenous hydration preceding, during and post-drug administration. Cardiotoxicity, myelosuppression, nephrotoxicity, and nausea/vomiting may also be observed.

6.7 Experimental Therapies

A potential promising new GVHD preventative agent is the histone deacetylase (HDAC) inhibitor, vorinostat. Lower doses of the drug appear to positively alter the balance of helper and regulatory T cells, reduce IL-6 and IL-12 production, and control dendritic cell activity (Holtan and Weisdorf 2017). Initial trials demonstrated efficacy and safety when vorinostat was paired with MMF and tacrolimus (Choi et al. 2014b). Side effects include hepatic toxicity, electrolyte abnormalities, QTc prolongation, mucositis, and an elevated risk of bacterial infection.

An analog of CTLA-4, Abatacept, inhibits T cell activation by blocking the co-stimulatory signal delivered between antigen presenting cells and T lymphocytes.

Additional studies are needed, but early results, particularly with non-hematologic transplant indications, have shown a benefit (Khandelwal et al. 2021; Ngwube et al. 2020). Infection risk is potentially less than other therapies, but remains present, especially when concurrent immunosuppressive therapy is used.

Alternative immunosuppressive/immune-modulatory mechanisms that have shown some benefit in the prevention of GVHD include CCR5 blockade via Marviroc (Moy et al. 2017) and inhibition of dipeptidyl peptidase 4 (DPP-4) by Sitagliptin (Farag et al. 2021; Martin 2021).

7 Acute GVHD Treatment

7.1 Corticosteroids

Systemic corticosteroids (starting at 1–2 mg/kg/day) are the backbone of therapy for acute GVHD grade II or higher as well as for those suffering from moderate to severe chronic GVHD. Once symptoms stabilize or improve, corticosteroids are then weaned slowly as tolerated (Gatza et al. 2020). Enteral corticosteroids, such as budesonide and beclomethasone, can be used in the setting of acute GI GVHD. The immunosuppressive effects of high-dose systemic and aberrantly absorbed local corticosteroids are numerous and include impaired antibody production, reduced T cell proliferation, increased proapoptotic lymphocyte activity, and alterations in leukocyte chemotaxis & anergy. Long-term exposure increases the risk of various opportunistic organisms, including DNA viruses (CMV, adenovirus, EBV and HHV-6), molds, and *Pneumocystis jiroveci (*Youssef et al. 2016*)*. Pneumocystis prophylaxis with pentamidine (inhaled or intravenous) or sulfamethoxazole-trimethoprim (following full hematologic count recovery) is thus imperative. Mold prophylaxis, such as micafungin, posaconazole, or voriconazole, may reduce the risk of serious disseminated fungemia. Hypertension, especially in the setting of additional calcineurin inhibitor usage, may necessitate treatment. Drug-induced hyperglycemia, metabolic syndrome, and hepatic cirrhosis can be seen. Finally, significant musculoskeletal side effects, including muscle atrophy and avascular necrosis, as well as psychological effects, such as irritability and insomnia, are observed with prolonged usage.

7.2 Ruxolitinib

In those with steroid-resistant GVHD, there is growing evidence that the JAK1/2 inhibitor, ruxolitinib, is superior to additional second-line agents with good tolerance and excellent response rates (Zeiser et al. 2020). Down-regulation of the JAK-STAT pathway leads to reduced inflammatory cytokine production and subsequent inhibition of CD4 T cells, DCs, and NK cells. Following drug initiation, cytopenias (most prominently thrombocytopenia and anemia), transaminitis, and elevations in cholesterol/triglycerides may be seen. Infectious risks include viral

reactivation, bacteremia, and fungal disease (Zeiser et al. 2020; Maschmeyer et al. 2019).

7.3 Tumor Necrosis Factor (TNF)-Inhibitors

Tumor necrosis factor (TNF) inhibitors, such as infliximab and etanercept, reduce the response to TNFα, which is an inflammatory cytokine associated with aGVHD (Salomon et al. 2018; Holler et al. 1990). Etanercept in addition to corticosteroid therapy may be effective for treating acute and chronic GVHD (Levine et al. 2008; Chiang et al. 2002). Acute and delayed infusion reactions can be seen with delayed reactions manifesting similarly to serum sickness. TNF inhibition is associated with an increased risk of opportunistic fungal, bacterial, and mycobacterial infections. Hepatitis and zoster reactivations may additionally occur (Henrickson et al. 2016).

7.4 Alemtuzumab

Severe steroid-refractory aGVHD may necessitate treatment with the CD52 targeting agent, Alemtuzumab (Schnitzler et al. 2009). While often effective in improving aGVHD, alemtuzumab causes prolonged, profound lymphopenia that places the patient at an elevated risk of systemic bacterial and fungal infections, including aspergillosis. Worsening of underlying viral illnesses or viral reactivation may additionally be seen. Infusion-related reactions and thyroid disease are possible adverse reactions. Alemtuzumab has also been trialed as a GVHD preventative therapy prior to allogeneic transplantation. Prophylactic alemtuzumab reduced GVHD incidence and severity, but this was at the expense of increased rates of graft failure, delayed immune reconstitution, and increased rates of relapse. More favorable outcomes were observed when incorporated into non-malignant disease conditioning regimens (Gatza et al. 2020).

7.5 Pentostatin

The purine analog, pentostatin, may be effective for steroid-refractory aGVHD by inhibiting T cell proliferation (Bolaños-Meade et al. 2005). Just as with other immunosuppressive medications, pentostatin is associated with an increased risk for infection. With regard to cytopenias, pentostatin is primarily associated with lymphopenia. Renal, hepatic, and neurologic toxicities are possible, especially with high doses. Pulmonary dysfunction can be severe but occurs most often with concurrent use of fludarabine, thus dual therapy with these medications during conditioning is not recommended.

7.6 Interleukin-2 Receptor (CD25-Alpha) Antibodies

The cytokine interleukin-2 (IL-2) plays an important role in stimulating pro-inflammatory T lymphocyte pathways and thus blockage of the IL-2 receptor via basiliximab or daclizumab can be effective in the prevention of GVHD. Trials testing these agents for treatment of acute GVHD were less promising (Gatza et al. 2020; Ross and Cantrell 2018). Overall, infectious complications were lower for these agents compared to other lymphocyte-targeting therapies, but an elevated risk of viral infections still appears to be present (Henrickson et al. 2016).

7.7 Brentuximab

Brentuximab, an anti-CD30 antibody, which is predominantly used in the treatment of classical Hodgkin lymphoma, showed a 24% partial response and 15% complete response rate in steroid-refractory acute GVHD (Chen et al. 2017b). Neutropenia is often observed with frequent dosing (weekly). Acute pancreatitis, neuropathy, hyperglycemia, infusion-related reactions, and hepatotoxicity may be seen. Despite targeting CD30-positive T lymphocytes, immunologic consequences (besides the mentioned neutropenia) appear to be less significant than those seen with other lymphocyte-targeting drugs (Maschmeyer et al. 2019).

7.8 Tocilizumab

In those experiencing cytokine release syndrome as a result of chimeric antigen receptor T cell (CAR-T) therapy, the IL-6 receptor directed monoclonal antibody, Tocilizumab, can be extremely effective in reducing severe systemic inflammation (Si and Teachey 2020). Early phase clinical studies showed promise in prevention of GVHD and treatment of acute and chronic GVHD (Drobyski et al. 2011; Kennedy et al. 2014). However, a recent phase III randomized double blind clinical trial reported nonsignificant trends toward reduced incidence of grade II-IV acute GVHD in recipients of HLA-matched unrelated donors, but no improvements in long-term survival (Kennedy et al. 2021). The drug appears to be associated with elevated rates of respiratory tract and cutaneous infections, in addition to therapy-induced neutropenia and mycobacterium reactivation (Henrickson et al. 2016). Non-immunologic/hematologic adverse drug events include increased serum cholesterol levels, transaminitis, infusion-related reactions, and hypertension.

7.9 Vedolizumab

Vedolizomab is a monoclonal antibody that works by blocking α4β7 integrin on T cells, thereby decreasing T cell trafficking to the gastrointestinal tract. Further efficacy and safety data regarding vedolizumab are needed, but the drug may be

particularly helpful in those suffering from severe gastrointestinal aGVHD (Fløisand et al. 2019). Given its GI-specific mechanism of action, vedolizumab appears to not have a significant association with serious opportunistic infections; although, *Clostridium difficile* disease may be seen (Ng et al. 2018).

7.10 Additional Immunosuppression Medications for Non-GVHD Indications

Further immunosuppressive therapies may be used to treat additional post-HSCT complications, including immune-mediated cytopenias, thrombotic microangiopathy, and idiopathic pneumonia syndrome.

Cytopenias that develop post-autologous or allogeneic HSCT due to varying types of immune dysregulation are associated with significant morbidity or morality. All cell lines may be affected. Other than blood product transfusions, immunosuppressive agents may be utilized. Corticosteroids and intravenous immunoglobulins may be inadequate requiring the use of second-line agents, including drugs targeting T cell dysfunction and B cell-driven antibody production (Michniacki et al. 2019).

Thrombocytopenia and microangiopathic hemolytic anemia secondary to endothelial damage from excessive complement system activation can lead to post-transplant thrombotic microangiopathy. Treatment with blockade of the terminal complement component C5 via eculizumab has been shown to be efficacious (Obut et al. 2016).

As noted above, TNF inhibition may be used in those with steroid-refractory GVHD. Additionally, etanercept and infliximab have shown benefit in those with idiopathic pneumonia syndrome (IPS) (Thompson et al. 2017; Panoskaltsis-Mortari et al. 2011). IPS typically presents within the first 100 days post-transplant as diffuse alveolar injury without apparent respiratory tract infection. Without treatment, the condition has a high mortality rate.

7.11 Rituximab

The anti-CD20 monoclonal antibody, Rituximab, targets B lymphocytes and has been utilized for various hematopoietic stem cell transplantation related indications, including to treat immune-mediated post-transplant cytopenias (Michniacki et al. 2019), and in an attempt to reduce chronic GVHD incidence by suppressing allogeneic donor B cell immunity (Arai et al. 2012). Given the propensity for EBV to target B lymphocytes, rituximab is also used to treat post-transplant EBV viremia/reactivation (Poppiti et al. 2016). Transient hypogammaglobulinemia may occur in patients following treatment. In addition, a small subset of patients may have persistent B cell lymphopenia resulting in prolonged hypogammaglobulinemia. Hepatitis B reactivation and progressive multifocal leukoencephalopathy have rarely been described (Henrickson et al. 2016). Fever and infusion-related hypersensitivity

may occur but can be prevented with pre-infusion acetaminophen, diphenhydramine, and/or corticosteroid administration.

7.12 Bortezomib

The powerful proteasome inhibitor, Bortezomib, should be considered in treatment-resistant post-transplant immune-mediated cytopenias. By targeting plasma cells, the drug reduces production of antibodies directed against hematologic cells (Michniacki et al. 2019). Those receiving bortezomib should be monitored closely for peripheral neuropathy, posterior reversible leukoencephalopathy syndrome, hepatotoxicity, cardiac dysfunction, herpes zoster reactivation, and gastrointestinal issues, including diarrhea and vomiting.

7.13 Eculizumab

Inactivation of the terminal complement component CD5 by eculizumab can lead to a drastic improvement in patients suffering from post-transplant TMA (Obut et al. 2016). With complement suppression, the drug greatly increases the risk of meningococcal disease. Those receiving Eculizumab are thus recommended to receive immunizations targeting all serotypes of meningococcus prior to drug administration; although, this may not be feasible in the post-transplant setting. Routine antibacterial prophylaxis against encapsulated organisms is also administered to patients while receiving eculizumab (Henrickson et al. 2016).

8 Conclusions

Immune suppression is used in allo-HSCT to prevent graft rejection, prevent GVHD, treat GVHD, and treat a number of other post-HSCT immune-related complications. Many of these approaches are based on the rich knowledge of aGVHD immunobiology worked out in murine models and tested in clinical trials. The primary immune suppression strategy used for GVHD prophylaxis remains CNI-based, but newer promising approaches including PTCy, co-stimulatory receptor blockade, and HDAC inhibition may soon also become standard of care. The primary immune suppressive treatment for GVHD remains corticosteroids, but JAK inhibition with ruxolitinib is emerging as the preferred second-line therapy. As with all immune suppressive therapies, patients must be closely monitored for on- and off-target side effects. These side effects need to be balanced with the need to treat the underlying disorder. Much remains to be learned about the complex immunobiology of aGVHD, SR-aGVHD, and cGVHD. Advances in these areas will yield more effective and less toxic therapies in the future.

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Immunosuppression in Multiple Sclerosis and Other Neurologic Disorders

Kaitlyn Koenig Thompson and Stella E. Tsirka

Contents

Abstract

Multiple sclerosis (MS) is an autoimmune disease of the central nervous system (CNS) characterized by peripheral immune cell infiltration into the brain and spinal cord, demyelination, glial cell activation, and neuronal damage. Currently there is no cure for MS, however, available disease-modifying agents minimize inflammation in the CNS by various mechanisms. Approved drugs lessen severity of the disease and delay disease progression, however, they are still suboptimal as patients experience adverse effects and varying efficacies. Additionally, there is only one disease-modifying therapy available for the more debilitating,

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progressive form of MS. This chapter focuses on the presently-available therapeutics and, importantly, the future directions of MS therapy based on preclinical studies and early clinical trials. Immunosuppression in other neurological disorders including neuromyelitis optica spectrum disorders, myasthenia gravis, and Guillain-Barré syndrome is also discussed.

Keywords

Autoimmunity · Disease-modifying therapies · Guillain-Barré syndrome · Immunosuppression · Multiple sclerosis · Myasthenia gravis · Neuromyelitis optica

1 Introduction

Multiple sclerosis (MS) is a chronic, demyelinating autoimmune disease of the central nervous system (CNS), affecting approximately 2.5 million people worldwide (Reich et al. 2018; Trapp and Nave 2008). The condition affects females more often than males (Reich et al. 2018; Dendrou et al. 2015) and though the etiology is still poorly understood, it is thought that both genetic and environmental factors play a causative role in the development of MS (Reich et al. 2018; Hauser and Oksenberg 2006). Clinical symptoms of the disease include disturbances in motor function, vision, and speech, fatigue, acute/chronic pain, and in severe cases, paralysis and cognitive impairment. Symptoms are caused by multifocal lesions in the brain and spinal cord that consist of inflammation, demyelination, blood-brain barrier (BBB) breakdown, peripheral immune cell infiltration, reactive gliosis, loss of oligodendrocytes, and axonal degeneration (Dutta and Trapp 2011; Trapp and Nave 2008).

MS is a heterogeneous condition consisting of different presentations and varying disease courses. Despite this, MS has been broadly categorized into subtypes: approximately 85% of patients are diagnosed with relapsing-remitting MS (RRMS) where symptomatic flare-ups, or relapses, are followed by periods of varying degrees of recovery. In majority of RRMS cases (~80%), patients progress to experience gradual worsening of relapses and fewer periods of recovery, termed secondary progressive MS (SPMS). A smaller fraction of patients experience progressing symptoms from the time of disease onset, a pattern recognized as primary progressive MS (PPMS). And yet another small subset of patients experience benign MS, where relapses are mild compared to RRMS and SPMS does not develop (Trapp and Nave 2008; Ransohoff et al. 2015; Hemmer et al. 2002).

As expected by the heterogeneity of its presentation and various forms, MS is defined by pathological alterations involving numerous cells types, both immune and non-immune. The primary pathological hallmarks of MS are areas of demyelination (referred to as “plaques” or “lesions”) in the white and gray matter of the brain and/or spinal cord. Demyelination is mediated by both innate and adaptive immune cells. Though the CNS is normally considered an “immune-privileged” site due to

the multicellular vascular blood-brain barrier (BBB), disruption of the BBB is apparent in all clinical subtypes of MS. This disruption allows peripheral immune cells to infiltrate the brain/spinal cord tissue.

T and B lymphocytes seem to be selectively recruited to the CNS by myelin autoantigens in MS and various hypotheses exist as to what triggers this recruitment. A CNS *intrinsic* model hypothesizes that events within the CNS result in the release of autoantigens into the periphery. On the other hand, an *extrinsic* model suggests that a peripheral insult, such as a system infection, leads to an aberrant immune response against myelin (Thompson et al. 2018).

Historically, MS has been considered a primarily T-cell-mediated disease with both CD4+ and CD8+ T cells present in MS lesions. CD4+ T helper cells typically predominate in acute lesions, whereas CD8+ cytotoxic T cells are found in chronic plaques (Chitnis 2007). B cells, on the other hand, are only recently becoming recognized as drivers of MS pathology. B cells produce antibodies that recognize various myelin epitopes and can also serve as antigen-presenting cells (APCs), communicating with T cells (Sospedra 2018). B cells can polarize T helper cells by secreting cytokines. Specifically, B-cell production of interleukin-6 (IL-6) seems to drive the autoimmune process by inhibiting the conversion of conventional T cells into regulatory T cells (Tregs) which are capable of immune suppression (Korn et al. 2008).

Cells of the innate immune system also infiltrate the CNS. Studies in MS animal models have implicated blood-derived monocytes as drivers of MS pathology, though it has been difficult to dissect their roles compared to the CNS resident innate immune cells, microglia. Both cells have been characterized to possess both harmful and beneficial functions as they can both secrete inflammatory cytokines and chemokines, but they can also produce growth factors and phagocytose myelin debris, a major obstacle to remyelination (Kotter et al. 2006). Studies on MS brain samples have shown that activated microglia in plaque regions express high levels of major histocompatibility complex class II (MHC II) molecules, suggestive of increased and active antigen presentation, stimulating the adaptive immune system and worsening the disease process (Boyle and McGeer 1990; Zhang et al. 2011; Raivich and Banati 2004). Other studies in animal models have suggested that the infiltrating monocyte-derived macrophages are the main drivers of pathology (Ajami et al. 2011; Yamasaki et al. 2014).

Although MS is probably the most well-recognized autoimmune disease of the CNS, there are several other neurological conditions with an autoimmune component, requiring pharmacological immunosuppression. Here, we discuss both current strategies to dampen the autoimmune response in MS as well as other neurological conditions, including neuromyelitis optica spectrum disorders (NMOSD), myasthenia gravis (MG), and Guillain-Barré syndrome. Importantly, we highlight potential future immunosuppressive therapies that may further improve the clinical treatment of MS.

2 Current Strategies to Promote Immunosuppression in Multiple Sclerosis

Therapeutic management of MS currently relies on immunomodulation to dampen the autoimmune response occurring in the CNS. Available disease-modifying therapies (DMTs) can be sorted into broad classifications based upon their mechanism of immunosuppression: (1) pleiotropic immunomodulators, (2) drugs interfering with DNA synthesis and repair, (3) reagents that sequester peripheral leukocytes, and (4) reagents that deplete immune cells. There are also non-DMTs that are commonly used to control relapses in RRMS. Corticosteroids, such as high-dose intravenous methylprednisolone, are the first line of treatment for acute symptomatic exacerbations. A recent study reported that oral administration of high-dose methylprednisolone was similar in efficacy and safety compared to the intravenous route (Le Page et al. 2015). Orally administered medications are favorable not only for patient convenience, but also because phobia of needles, impaired dexterity, and reactions at injection sites often result in poor patient compliance (Mohr et al. 2001). In this section, we will discuss currently approved DMTs based upon their mechanisms of immune suppression (Fig. 1).

2.1 Pleiotropic Immunosuppressants

The first DMT, recombinant interferon-β (IFN-β), is a pleiotropic drug and remains a leading therapeutic option for RRMS since its approval by the United States Food and Drug Administration (FDA) in 1993. Its availability to patients marked a significant milestone in MS therapy as it was the first time the disease was viewed as treatable (Ransohoff et al. 2015). Different forms of the drug are now available including IFN-β1b (Betaseron, Betaferon, Extavia) and IFN-β1a (Avonex, Rebif, Plegridy), though IFN-β1b was the first to be studied and approved. In the first multicenter study of 372 RRMS patients, IFN-β1b was shown to reduce annual relapse rate by ~30% (Paty and Li 1993). Recently, an 11-year clinical study showed that early treatment with IFN-β1b in patients with clinically isolated syndrome (CIS; suggestive of a first MS attack) resulted in long-term benefits (Hartung et al. 2019). The recombinant cytokine binds the heterodimeric, multi-subunit IFN-β receptor (IFNAR1 and IFNAR2), resulting in Janus Activated Kinase-Signal Transducer and Activator of Transcription (JAK-STAT) signaling and its pleiotropic effects are the result of transcriptional effects on hundreds of genes (Hojati et al. 2016). The most prominent immunosuppressive actions of IFN-β include inhibition of T-cell activation through decreased expression of MHCII and co-stimulatory molecules, increased apoptosis of autoreactive T cells, and reduction in the stimulatory capacity of B cells (Dhib-Jalbut and Marks 2010). A cytokine shift has also been observed upon IFN-β treatment, inhibiting Th1 pro-inflammatory cytokines and promoting release of Th2 anti-inflammatory cytokines (Ersoy et al. 2005).

There are patients, however, who do not respond well to IFN-β and exhibit either severe side effects or no improvement in disease activity. Another pharmacological

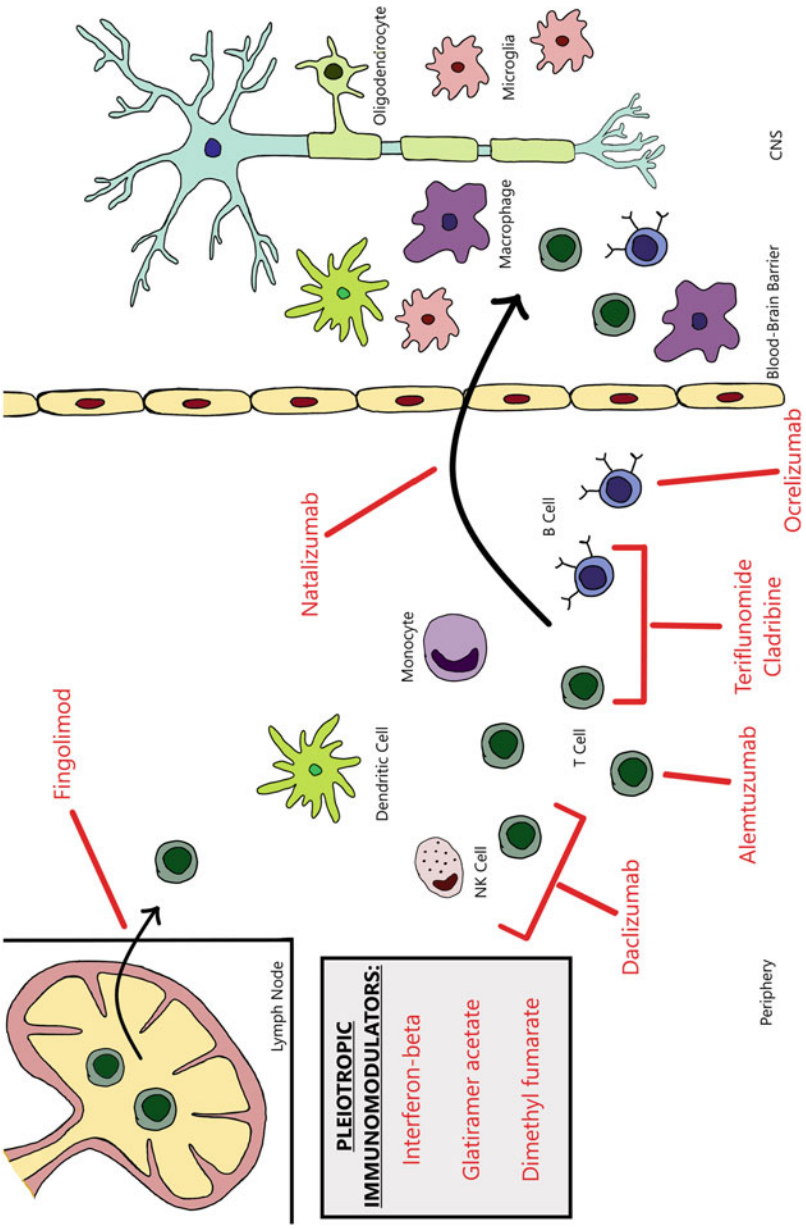

Fig. 1 Targets of currently approved DMTs (indicated in red) in the pathological setting of MS. DMTs with pleiotropic mechanisms are indicated in gray box

option became available in 1997 with the approval of glatiramer acetate (GA; Copaxone), a synthetic copolymer of amino acids analogous to an epitope of myelin basic protein (MBP). Interestingly, GA was discovered when Teitelbaum and colleagues sought to produce a synthetic antigen capable of inducing experimental autoimmune encephalomyelitis (EAE), the primary animal model of MS. Surprisingly, rather than inducing disease, GA protected against EAE induction (Teitelbaum et al. 1971). In clinical trials, GA reduced the relapse rate and was relatively well-tolerated in humans (Johnson et al. 1995; Comi et al. 2009). It also displayed comparable efficacy to IFN-β formulations. A major mechanism of action of GA is the induction of apoptosis in CD4+ T cells, and a recent study in RRMS patients suggests that this is a biomarker of optimal treatment response (Boziki et al. 2019). GA was shown to increase the number of anti-inflammatory monocytes and immunosuppressive Tregs, maintaining these effects over a decade of GA administration (Spadaro et al. 2017).

Dimethyl fumarate (DMF, Tecfidera), another pleiotropic drug, was approved as a first-line treatment for RRMS in 2013. DMF activates the transcription factor, nuclear factor (erythroid-derived 2)-like 2 (Nrf2), which is responsible for maintaining cellular redox homeostasis. When transported to the nucleus, Nrf2 induces expression of antioxidants and detoxifying enzymes (Ma 2013). DMF also modulates Nrf2-independent pathways. For instance, the agent suppresses NF-κB signaling, resulting in the reduction of inflammatory cytokines and induction of Th2, anti-inflammatory phenotypes (Gillard et al. 2015). Importantly, a recent study reported persistent changes in both the innate and adaptive immune system in MS patients after 12 months of DMF treatment, observing a decrease in effector memory T cells, memory B cells, and expression of antigen presentation molecules (Montes Diaz et al. 2018).

2.2 Drugs Interfering with DNA Synthesis/Repair

Mitoxantrone (Novantrone) was initially approved as an antineoplastic agent as it globally disrupts DNA synthesis through inhibition of type II topoisomerase (Shenkenberg and Von Hoff 1986). Mitoxantrone is generally immunosuppressive, and is only prescribed in cases of rapidly worsening MS. Although a multicenter study of patients with severe and worsening RRMS or progressive MS showed that mitoxantrone did reduce progression of disability (Hartung et al. 2002), its use in MS has dramatically decreased due to severe side effects, such as cardiac toxicity and acute leukemia (Capobianco et al. 2008), and the approval of less dangerous medications.

Teriflunomide (Aubagio) is an oral inhibitor of dihydroorotate dehydrogenase (DHODH), a mitochondrial enzyme necessary for de novo pyrimidine synthesis. Inhibition of this enzyme limits availability of pyrimidines in proliferating T and B cells, reducing the number of autoreactive lymphocytes available to cross the BBB (Claussen and Korn 2012). Three large phase III trials showed that 7–14 mg of teriflunomide decreased annual relapse rates and MRI disease activity, which

resulted in the approval of the drug in 2004 (O'Connor et al. 2011). A more recent 9-year follow-up study showed that long-term treatment remains efficacious and is well-tolerated in patients (O'Connor et al. 2016).

Cladribine (Mavenclad), a synthetic chlorinated deoxyadenosine analog, is the most recent drug approved by the FDA for MS. Cladribine is taken up by cells, and undergoes several phosphorylation steps to produce the active compound, 2-chlorodeoxyadenosis 5′-triphosphate (2-CdATP). 5′-nucleotidases in most cells degrade 2-CdATP, however, lymphocytes have lower levels of these enzymes and higher levels of deoxycytidine kinase (DCK), the enzyme responsible for cladribine phosphorylation. This ultimately results in intracellular accumulation of 2-CdATP selectively in lymphocytes, and the active compound becomes incorporated into DNA, leading to strand breaks and cell death (Leist and Weissert 2011; Baker et al. 2019). In comparison with monoclonal antibodies that deplete B cells, such as ocrelizumab and rituximab, cladribine's mode of action results in a more gradual depletion (Montalban et al. 2017; Baker et al. 2019). A recent study showed that after 20 days of oral treatment, CD19+ B cells and CD8+ T cells return to baseline levels, and patients maintain no clinical or MRI disease activity. Further, monocyte and neutrophil numbers remain intact resulting in less risk of opportunistic infections (Comi et al. 2019).

2.3 Reagents That Sequester Peripheral Leukocytes

Fingolimod (FTY720; Gilenya), which reduces CNS inflammation by limiting lymphocytes in the periphery, was the first oral medication for RRMS patients. Approved by the FDA in 2010, fingolimod is a sphingosine-1-phosphate (S1P) receptor antagonist that prevents T- and B-cell egress from lymph nodes, reducing the number of autoreactive lymphocytes in the CNS. A phase III study reported that oral treatment with fingolimod for 12 months was superior to intramuscular IFN-β1a in terms of annualized relapse rate and MRI disease activity (Cohen et al. 2010).

Natalizumab (Tysabri) is a humanized monoclonal antibody against the α4 subunit of the very late antigen 4 (VLA4) integrin expressed on leukocytes. Blockage of this cell adhesion protein functions to prevent lymphocyte migration into the CNS as it blocks interaction with vascular-cell adhesion molecule 1 (VCAM-1) on vascular endothelial cells in the brain and spinal cord (Ransahoff 2007). Natalizumab was studied as both a monotherapy and an IFN-β therapy. Both phase III clinical trials took place over the course of 2 years and included only RRMS patients. As a monotherapy, natalizumab reduced relapse rate and gadolinium-enhancing lesions on MRI at year 2 by 92% (Polman et al. 2006). When natalizumab was administered to patients on IFN-β, who had at least one relapse during the past year of treatment, the combination of the drugs was observed to be significantly more effective than interferon alone (Rudick et al. 2006). The use of natalizumab is limited by the occurrence of progressive multifocal leukoencephalopathy (PML), a fatal brain infection, and current studies are

attempting to establish biomarkers to predict the risk of PML in MS patients (Schwab et al. 2013, 2016).

2.4 Reagents Depleting Immune Cells

Ocrelizumab (Ocrevus) is an anti-CD20 antibody, acting to deplete CD20-expressing B cells. The approval of this agent was groundbreaking in the field of MS therapeutics as it was the first drug to show efficacy for patients with PPMS (Mulero et al. 2018). A phase III placebo-controlled trial of 732 PPMS patients reported that those receiving ocrelizumab displayed lower rates of progression (assessed clinically and by MRI) compared to the placebo group (Montalban et al. 2017). The remarkable results of the anti-CD20 therapy have renewed interest in the role of B cells in MS pathology, as MS has historically been considered a primarily T-cell-mediated disease.

Alemtuzumab (Lemtrada), a humanized monoclonal antibody targeting CD52 on lymphocytes, monocytes, granulocytes, and natural killer (NK) cells induces rapid lymphopenia through antibody-dependent cellular cytotoxicity (ADCC). Clinical studies showed that infusion of alemtuzumab decreased annualized relapse rate, reduced disability progression, and reduced MRI disease activity. Further, it was observed to be superior to IFN-β1a therapy (Coles et al. 2012; Cohen et al. 2012). The most common adverse effect is secondary autoimmunity, most typically involving the thyroid gland. A long-term follow-up study confirmed that alemtuzumab stabilizes disease in patients with highly active RRMS (Tuohy et al. 2015).

Daclizumab (Zinbryta) is a humanized monoclonal antibody against the CD25 subunit of the interleukin-2 (IL-2) receptor, highly expressed on activated T cells. This results in functional impairment of the T cells. Daclizumab treatment results in a decrease in circulating CD4+ and CD8+ T cells and expansion of $CD56^{bright}$ natural killer (NK) cells, which is considered an immunoregulatory NK cell population due to cytokine profiles and expansion during states of immune tolerance (Bielekova et al. 2006). Daclizumab approved in 2016 is prescribed only to patients who are refractory to at least two first-line treatments (Baldassari and Rose 2017). Interestingly, a phase II study that added daclizumab therapy on to IFN-β treatment found that the combination may more effectively reduce disease activity compared to IFN-β alone (Wynn et al. 2010).

3 Looking Ahead: The Future of Immunosuppressive Therapies for Multiple Sclerosis

Though there has been truly amazing progress in the field of MS therapeutics over the past decade, the limitations of currently available agents justify continued research efforts to improve treatment options for patients. Available immunotherapies are variable in their efficacies, often produce adverse effects, and ultimately are unable to prevent disease progression. Although there is an abundance

of preclinical, and some clinical, focus on addressing these limitations by studying agents capable of promoting remyelination and repair, here, we will focus solely on innovative approaches to improve therapies that modulate the immune system in MS.

3.1 Targeting B Cells

Recently, B cells have gained attention as an exciting and potentially more effective therapeutic target in various subtypes of MS due to the success of ocrelizumab, and other B-cell-targeted antibodies in clinical trials (namely, rituximab and ofatumumab). Other agents that modulate B cells by various mechanisms are likely to enter the clinic and be approved in the coming years.

Early clinical studies have reported positive results in RRMS patients treated with Bruton's tyrosine kinase (BTK) inhibitors. BTK, a non-receptor tyrosine kinase, regulates B-cell function, playing a central role in B-cell receptor (BCR) signaling. BTK signaling pathways also modulates myeloid cells. BTK inhibitors have been available in recent years for the treatment of B-cell leukemias/lymphomas (Liang et al. 2018). Now, newer and more selective inhibitors have been developed and are currently being investigated in not only B-cell malignancies, but also in autoimmune settings, such as rheumatoid arthritis and MS (Zhang et al. 2018). A phase II clinical trial reported that after 24 weeks of once daily oral treatment with 75-mg of the BTK inhibitor, evobrutinib, RRMS patients displayed decreased gadolinium-enhancing lesions on T_1-weighted MRI compared to patients receiving placebo (Montalban et al. 2019). A phase III study has been posted to compare evobrutinib's effectiveness to the current first-line treatment, IFN-β1a (NCT04032171).

Another B-cell-directed therapeutic target under investigation is B-cell-activated factor (BAFF). BAFF is a member of the tumor necrosis factor family which promotes B-cell development and survival. It has been observed to be elevated in the cerebrospinal fluid (CSF) of MS patients (Ragheb et al. 2011) as well as accumulate in inflammatory demyelinating brain lesions (Krumbholz et al. 2005). A humanized recombinant fusion protein, Atacicept, was developed to block both BAFF and a proliferation-inducing ligand (APRIL), which is also involved in B-cell differentiation and maturation signaling. After preclinical work showing a decrease in mature B cells (Gross et al. 2001), a phase II trial also showed that Atacicept treatment did reduce serum immunoglobulin and number of circulating mature B cells in RRMS patients, however, there was an unexpected increase in relapses. The cellular and symptomatic effects did revert to that of the placebo group after discontinuation of the drug, illustrating reversibility of the mechanism (Kappos et al. 2014). VAY736, a humanized monoclonal antibody against one of the receptors for BAFF (BAFF-R) has also been evaluated in RRMS patients in a phase II trial, however, results are not yet posted (NCT02038049).

3.2 Stem Cell Therapies

Hematopoietic stem cells (HSCs) are the primary stem cell population of the bone marrow, capable of giving rise to all types of blood cells. Hematopoietic stem cell transplantation (HSCT) has long been used as a method to treat hematological malignancies, but only in the early 1990s was it considered for use in MS patients after pivotal preclinical studies (Karussis et al. 1992, 1993). It is important to note that stem cell transplant is a high-risk procedure with aggressive immunoablation to extinguish pathogenic immune cells and "reset" the immune system with only HSCs. Typically, a patient's own HSCs are employed (autologous HSCT; aHSCT) (Karussis and Petrou 2018).

A multicenter phase II trial showed that aHSCT in MS patients with poor prognosis (both RRMS and SPMS subtypes) led to long-lasting remission in the majority of patients with no DMT regimen. Further, there was significant neurological improvement as the rate of brain atrophy decreased to that of healthy aging controls (Atkins et al. 2016). A larger-scale study also reported that approximately half of the patients undergoing HSCT did not exhibit neurological progression 5 years post-transplant. Successful outcomes were associated with younger age, an RRMS subtype, and fewer previous immunotherapies (Muraro et al. 2017). Though small, a recent study in Sweden showed that five out of ten patients exhibited sustained remission 10 years after aHSCT, and the investigators suggested that MS was "resolved" (characterized by normalized intrathecal IgG production and CSF neurofilament light levels) in three out of the five patients (Tolf et al. 2019). Though exciting results continue to be obtained, the risks of the procedure remain a concern and thus, development of new, safer protocols is required to consider this a standard therapy.

Mesenchymal stem cells (MSCs) are another cell therapy currently explored for severe cases of MS. MSCs are stromal precursor cells which, in the bone marrow, function to support hematopoiesis and display highly anti-inflammatory properties, inhibiting lymphocyte and APC function and modulating T-regulatory cells (Karampera et al. 2003; Corcione et al. 2006; Di Ianni et al. 2008; Beyth et al. 2005). In preclinical studies using the EAE model, MSC transplant therapy is reported to not only be anti-inflammatory, but also neuroprotective, supporting remyelination of damaged axons (Kassis et al. 2008; Zappia et al. 2005). In clinical studies of MS patients, bone marrow-derived MSC administration was observed to be generally well-tolerated, but small sample sizes limited conclusions concerning efficacy of the treatment (Yamout et al. 2010; Bonab et al. 2012). Recently, the Mesenchymal Stem cells for Multiple Sclerosis (MESEMS) study group published their protocol for a larger-scale phase I/II study that aims to evaluate the safety and activity of intravenous autologous bone marrow-derived MSCs in patients with RRMS, SPMS, and PPMS (Uccelli et al. 2019). Additionally, studies have evaluated the safety and efficacy MSC-derived neural progenitors, which were shown to be neurotrophic and immunoregulatory. A phase I trial of MSC-derived neural progenitors administered intrathecally to patients with progressive MS showed that the treatment was well-tolerated with only minor adverse events occurring. Further,

evidence of clinical disability trended towards improvement following treatment (Harris et al. 2018).

4 Immunosuppressants for Other Neurologic Disorders

4.1 Neuromyelitis Optica Spectrum Disorders

Neuromyelitis optica spectrum disorders (NMOSD) is a group of relapsing neuroinflammatory diseases distinct from MS in its pathophysiology and thus, the approach to immunosuppression also differs (though there are some commonalities). Progression is rare in NMOSD, but relapses are very severe and characterized by complete vision loss and/or extreme motor/sensory dysfunctions as a result of inflammatory lesions formation in the spinal cord (Wingerchuk and Weinshenker 2003). NMOSD is typically distinguished from other CNS autoimmune disorders by the presence of an IgG autoantibody against the water channel, aquaporin 4 (AQP4) (Papadopoulos and Verkman 2012), though not all patients are anti-AQP4 positive. First-line therapy for acute relapse of NMOSD is comprised of corticosteroid treatment (typically high-dose intravenous methylprednisolone). Plasma exchange is the next option for progressive or refractory conditions (Kleiter et al. 2018). NMOSD relapses are disabling with patients rarely experiencing full recovery, therefore, at least 5 years of maintenance immunotherapy is standard, with the intent of preventing relapses and accumulation of disability (Patterson and Goglin 2017).

Chronic low-dose corticosteroids are one option to prevent NMOSD attacks, usually in combination with another immunosuppressive. However, long-term use of corticosteroids often results in adverse effects such as hyperglycemia, hypertension, and osteoporosis (Kleiter and Gold 2016). Azathioprine, a purine antagonist that acts to inhibit DNA synthesis, has been found to be effective in long-term treatment of NMOSD (either with or without prednisone), more so than steroid therapy alone (Costanzi et al. 2011; Mandler et al. 1998; Bichuetti et al. 2010). Another option is mycophenolate mofetil, a drug that is indicated for psoriasis and renal transplant rejection, but is also often employed in NMOSD as well. Mycophenolate mofetil is a prodrug, the active metabolite being mycophenolic acid, which inhibits lymphocyte proliferation by preventing guanosine nucleotide biosynthesis (Mealy et al. 2014; Jacob et al. 2009). Mitoxantrone, previously discussed above as a therapeutic option for MS, has also been observed to be beneficial, reducing the annualized relapse rate in the first year of treatment in patients with highly relapsing NMOSD (Kim et al. 2011b). Interestingly the first-line therapy for MS, IFN-β, as well as the DMTs natalizumab and fingolimod, exacerbate NMOSD (Shimizu et al. 2008; Kleiter et al. 2012; Min et al. 2012).

B-cell depletion has become an obvious therapeutic strategy due to the presence of AQP4 autoantibodies in majority of NMOSD patients. Cree et al. showed that six out of eight patients were relapse-free after one year of treatment with rituximab, an anti-CD20 antibody (Cree et al. 2005). Other studies have confirmed the safety and efficacy of rituximab treatment with a modified protocol; rather than the standard

fixed maintenance therapy with rituximab every 6 months, the investigators only retreated with rituximab after determining whether the frequency of CD27+ memory B cells in peripheral blood of NMOSD patients exceeded 0.05% for the initial 2 years of treatment, and 0.1% thereafter. They observed a reduction in relapse rate and improvement of disability (Kim et al. 2011a, 2013). A more recent report assessed long-term (>7 years) treatment of NMOSD patients with the aforementioned treatment regimen. It was concluded that this long-term, modified approach was beneficial as no patients experienced serious side effects, there was a 97% reduction in annualized relapse rate compared to fixed treatment, memory B-cell population remained low, and unnecessary treatments with rituximab were avoided through the monitoring protocol (Kim et al. 2019).

Stem cell therapies are also under investigation for the treatment of severe, refractory NMOSD. A small study of two patients reported disappearance of anti-AQP4 antibodies, reduction of spinal cord lesions, and clinical remission 3-years post-allogeneic HSCT. Interestingly, the patients from this study had previously undergone aHSCT, indicating that allogeneic stem cell transplant may be more effective than autologous (Greco et al. 2014). A phase II/III trial is currently recruiting NMOSD patients to test an aggressive, investigational aHSCT procedure after conditioning with rituximab, cyclophosphamide, and antithymocyte globulin, a rabbit polyclonal antibody to deplete lymphocytes (NCT03829566).

4.2 Myasthenia Gravis

Myasthenia gravis (MG) is an autoimmune disease of the neuromuscular junction in which autoantibodies interfere with nerve-muscle communication. Patients typically test positive for anti-acetylcholine receptor (AChR) antibodies. Of those who are negative for AChR autoantibodies, ~40% will harbor antibodies for muscle-specific tyrosine kinase (MuSK) (Tandan et al. 2017). Autoimmune disruption in nerve-muscle conduction manifests as muscle fatigue and weakness in MG patients. As with MS and NMOSD, the initial treatment is typically corticosteroids to suppress inflammation; however, long-term use is limited by adverse effects (Gotterer and Li 2016) and most patients do require long-term immunosuppression to remain in remission.

Both azathioprine and mycophenolate mofetil are commonly prescribed to MG patients, as well as NMOSD, as previously discussed. An important 1998 randomized, double-blind trial compared prednisolone alone with prednisolone plus azathioprine in MG patients. Here, they found that the addition of azathioprine to corticosteroid treatment was able to reduce the maintenance dose of prednisolone, reduce side effects, and reduce relapses over the course of 3 years (Palace et al. 1998). Mycophenolate mofetil was first reported to be rapidly effective in a case study of a 26-year-old MG patient whose symptoms were previously difficult to manage with other immunosuppressants (Hauser et al. 1998). A few years after the publication of this case report, a retrospective study reported efficacy, but a more delayed onset of action of mycophenolate mofetil in MG patients. The investigators

believe that since mycophenolate mofetil inhibits purine synthesis (preventing proliferation of lymphocytes), it does not kill pre-existing autoreactive lymphocytes, thus, the gradual death of the activated cells prior to treatment is what shows initial symptomatic improvement (Chaudhry et al. 2001).

Cyclosporine and tacrolimus (FK506), both calcineurin inhibitors which inhibit T-cell function by blocking the synthesis of interleukin-2 (IL-2) and interferon, are also often administered in MG cases as long-term immunosuppressants, allowing tapering/discontinuation of corticosteroids. However, cyclosporine use is often discontinued due to adverse effects, most often nephrotoxicity, that occur over time (Ciafaloni et al. 2000). Although tacrolimus is more well-tolerated in comparison with cyclosporine, there are still incidences of side effects (Nagaishi et al. 2008; Minami et al. 2011).

Eculizumab was recently approved by the FDA for MG after a phase III trial (REGAIN) that showed that, though the agent didn't significantly improve the primary endpoint of MG-"Activities of Daily Living" Score, it did decrease exacerbations, need for rescue therapy, and hospital admissions. Eculizumab is a monoclonal antibody against the complement protein, C5, preventing formation of the terminal complement complex, C5b-9 (Howard et al. 2017).

Expectedly rituximab, as an off-label therapy, has also been observed to benefit patients with MG by depleting B cells and thus, decreasing levels of autoantibodies. A recent systematic retrospective analysis of the safety and efficacy of rituximab in MG patients reported that the agent was safe and effective for both AChR- and MuSK-positive MG patients, with a more robust response evident in the MuSK subset. Though, this study was unable to conclude what an optimal rituximab treatment regimen was comprised of due to the limitations associated with the reviewed case reports (Tandan et al. 2017). Another recent retrospective study assessed the long-term efficacy and safety of repeated treatments with low-dose rituximab in patients with severe, refractory MG. Here, it was reported that the repeated low-dose treatments, as guided by circulating CD19+ B-cell repopulation, was an effective therapy for difficult-to-manage MG (Choi et al. 2019). Further, a large nationwide study in Austria reported rituximab to be safe, rapidly efficacious, and provide the greatest benefits in MuSK-positive MG patients (Topakian et al. 2019). Rituximab is not without limitations, however, as severe adverse events, notably the development of PML, can occur.

4.3 Guillain-Barré Syndrome

Guillain-Barré syndrome (GBS) is an acute autoimmune disease resulting in demyelination of the peripheral nerves. It is characterized by immunoglobulin and complement-mediated attack on axons, as well as T cell and macrophage infiltration of peripheral nerves. Autoantibodies against gangliosides are often present in the serum of GBS patients and bind to Schwann cell surfaces, nodes of Ranvier, and peripheral axons (Ang et al. 2004). Patients experience rapid (on the scale of weeks), progressive weakness of the limbs, most often bilaterally, and with or without

involvement of respiratory muscles. It is believed that GBS is caused by infection, which induces an aberrant immune response against the peripheral nerves (Hughes and Cornblath 2005). In particular, there is an abundance of evidence drawing an association between *Campylobacter jejuni* infection and the development of GBS (Ang et al. 2004). Though the majority of patients improve without immunotherapy, it is believed that early immunosuppression can reduce disease severity and facilitate a quick recovery. Plasma exchange was the first therapy to show efficacy in a 1985 randomized trial (Group 1985) and is now considered the gold standard treatment (Hughes and Cornblath 2005), with randomized clinical trials and large-scale studies supporting its use (Chevret et al. 2017). There have been a number of clinical studies showing that intravenous immunoglobulin (IVIG) is effective as well (Hughes et al. 2014; Van der Meche 1992). In contrast to the previously discussed neuroinflammatory diseases, corticosteroids are ineffective in GBS (Hughes et al. 2016). Unfortunately, treatments to completely prevent (or reverse) lingering disability are still lacking, and the development of improved therapies is critical.

5 Conclusion

This review highlights current as well as potential up-and-coming immunosuppressive strategies for neurologic conditions. The past decade has been truly remarkable in availability of novel immunomodulatory options for patients with MS and other neuroinflammatory diseases. Though a number of drugs are now approved for MS, there is still a critical need for the characterization and development of agents that are capable of suppressing the immune system while limiting adverse effects. Further, the largest unmet need in the field of MS therapeutics is modulating the immune system to halt or, more ideally, reverse disease progression. Additionally, as evident by the more limited therapies available for NMOSD, MG, and GBS, these conditions are all in need of more therapeutic options for patients who experience severe, refractory disease. Overall, however, current immunosuppressive regimens have enabled the treatment of these disabling neurologic autoimmune diseases, in most cases slowing progression and improving the patients' quality of life. There is no doubt these strategies will continue to be refined, and new immunosuppressive approaches will be available in the future to enhance outcomes for these patients.

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Novel Immunosuppression in Solid Organ Transplantation

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Contents

Abstract

Solid organ transplantation and survival has improved tremendously in the last few decades, much of the success has been attributed to the advancements in immunosuppression. While steroids are being replaced and much of the

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immunosuppressive strategies focus on steroid free regimens, novel agents have introduced in the induction, maintenance, and treatment of acute rejection phase. MTOR inhibitors have helped with the renal sparing side effect from the calcineurin inhibitors, newer agents such as rituximab have decreased the incidence of donor-specific antibodies which led to decreased incidence of acute rejection reactions. In this chapter we discuss the newer therapies directed specifically for solid organ transplantation.

Keywords

Heart transplantation · Immunosuppression

1 Introduction

Heart transplantation is the gold standard treatment for end stage heart failure. The survival rates have progressively improved the last few years with the introduction to the evolving immunosuppressive medications to the point the 5-year survival rate is 69% (Lund et al. 2014). The success is primarily due to the various classes of medications being used for either induction or maintenance therapies. After any transplant, the recipient takes immunosuppressive medications throughout the length of their lifetime and is monitored for rejection with cardiac biopsies and laboratory blood checks. The risk of rejection always remains, alongside of heightened infectious risks, malignancies, and post-lymphoproliferative disorders.

After the first heart transplant in 1967, heart transplant lost its enigma owing to graft rejection and infection (Barnard 1967). When immunosuppression was first introduced, it was non-specific and broad, included mercaptopurines and corticosteroids (Mueller 2004). Azathioprine was introduced before steroids in the 1950s. Then came corticosteroids are an innate part of the immunosuppressive therapies after solid organ transplant, both in the induction and the maintenance phases in the 1960s. Anti-lymphocyte sera were introduced in the 1970s dominated in the use of induction therapy. Mercaptopurines such as azathioprine were the cornerstone of immunosuppression in the 1980s. After the introduction of calcineurin inhibitors i.e., tacrolimus and cyclosporine, they remained mainstay maintenance therapy since the 1980s. We then saw the emergence of newer medications like mycophenolate mofetil in the 2000s alongside of Sirolimus (Vera et al. 2017). The monoclonal antibodies were introduced later part of the decade with the latest being Belatacept which was FDA approved in 2011 for kidney transplantation. It was later also used after heart transplantation, although many patients in those studies were multi-organ transplant recipients (Launay et al. 2020). The biggest challenge for immunosuppression is to mitigate the harm in inducing malignancies and opportunistic infections, while providing adequate host-graft tolerance.

Induction therapy is controversial in heart transplantation. It is beneficial in patients who are at an increased risk of death from rejection and for patients with

Table 1 Classes of immunosuppressants studied for cardiac transplantation

1	Steroids	
2	Antimetabolites	Azathioprine
		Mycophenolate mofetil
		Mycophenolic acid
3	Polyclonal antibodies	Antilymphocyte globulin
		Antithymocyte globulin
		Muromonab (OKT3)
4	Monoclonal antibodies	Daclizumab
		Basiliximab
		Campath-1 H
		Rituximab
		CTLA-4-Ig
5	Calcineurin inhibitors	Cyclosporine
		Tacrolimus
6	Proliferation signal inhibitors	Sirolimus
		Everolimus
7	Lymphocyte modulation	FTY720
9	Other	IVIG
		Bortezomib
		Eculizumab

low risk of rejection, it causes more harm. For patients with long-term VAD support, African-American ethnicity and extensive HLA matching most likely benefit from induction therapy (Higgins et al. 2005). Not all cardiac transplants receive induction therapy, only 50% of them do. Early graft transplant reduced the frequency and severity of early graft rejection (Delgado et al. 2011). The treatment for induction therapy includes monoclonal antibodies or polyclonal antibodies combined with a calcineurin inhibitor drug. Minnesota antilymphocyte globulin (MALG) which is a polyclonal antibody was used in an experimental fashion even though it was not FDA approved in the 1980–1990s (Collins et al. 1996). Thymoglobulin was approved for widespread use in 1999, was derived from rabbit serum (Mahmud et al. 2010), and soon became the most popular agent used for induction therapy. The monoclonal antibodies which are new target the CD proteins on the T or B cells, especially CD3, CD25 and CD52 (Mahmud et al. 2010). UNOS registry studied patients from 1987 to 2012, and they saw that around 46% of patients among 8,216 were induced. Of the induced agents, 55% were IL-2Rab, 4% alemtuzumab, and 40% ALG/ATG/thymoglobulin (Whitson et al. 2015). Induction therapy should be highly individualized and needs a well-designed protocol. The types of immunosuppressants and the types of rejection reactions are outlined in Table 1 and Fig. 1.

Sensitization is seen around 10% of the heart transplants, the risk increases with the presence of an LVAD. Sensitization was defined as the incidence of panel reactive antibody (PRA) $\geq$10% with $\geq$1 a strong positive antibody. De-sensitization is defined as $\geq$25% reduction of fluorescence intensity $\geq$90% of

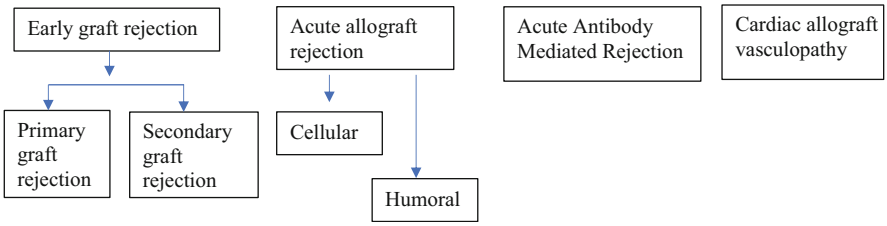

Fig. 1 Types of rejection

strong positive antibodies on follow-up PRA testing (Edwards et al. 2019). The newer regimens were introduced or studied to decrease in the intensity of sensitization.

The risk of acute rejection and allograft loss is greatest in the first three months after transplant. So it is inevitable for immunosuppression to be highest at this time and tapered to a maintenance level 6–12 months after (Tönshoff 2020). Most transplant centers use three drugs for continued immunosuppression. More than 80% patients are on two calcineurin inhibitors (Mudge Gilbert 2007). CAV affects 50% of heart transplant recipients after 5 years of transplant.

The newer immunosuppressants are monoclonal antibodies, calcineurin inhibitors, and other medications (Costanzo 2001). We chose to review the newer immunosuppressant medications for the heart transplant patients.

1.1 Monoclonal Antibodies

OKT3 is a murine antibody which acts against CD3 complex which binds to T cells. It increased the rate of infections, so newer agents like IL2 receptor antibodies and anti-thymocyte globulin have been the corner stone of induction therapy lately. Using the anti-IL2 antibodies effectively reduces the frequency and severity of early graft rejection, allows delay in calcineurin inhibitors introduction, and also does not have an increased incidence of infections (Delgado et al. 2011).

2 Basiliximab

The two commonly used anti-IL2 antibodies are: daclizumab and basiliximab. Basiliximab (BAS) is a monoclonal antibody that is chimeric against IL2 and has low immune response. Therefore it does not elicit a strong immune response (Delgado et al. 2011). SIMCOR registry showed that BAS had less incidence of fever, acute pulmonary edema, hypotension when compared to OKT3. Biopsy-proven cellular rejection was not significantly different (39.6% of basiliximab vs 40.4% of OKT3, $p = 0.87$) between the groups (Segovia et al. 2006). BAS was deemed to be safer and better tolerated than OKT3. Aguero et al. compared IL2 antagonists, cyclosporine, mycophenolate, and steroids among other regimens and

proved a strong association with that group with survival (Aguero et al. 2008). BAS can also confer renal protection if patients have a higher pre-operative creatinine value up to 6 months post-transplant when tested in seven patients with renal dysfunction (Delgado et al. 2005). When BAS was compared with ATG retrospectively, there was no difference between acute rejection and delayed graft rejection between the two groups, it had lower incidence of lung infection as well as reduction in cell counts (Wang et al. 2012). Forty-three patients who received BAS had a two-year survival rate of 92.86%, no severe adverse events and no rejection were observed (Chou et al. 2008). Mattei et al. reported that BAS had a composite safety end-point significantly lower than ATG (50.0% vs 78.6%, $p = 0.01$), and death due to infections was also less in the basiliximab group (Mattei et al. 2007). Acute rejection episodes were similar in both groups. BAS also reduced delayed graft function (DAF) when compared to other drugs in patients with high risk allografts (Fernandez Rivera et al. 2005). Induction therapy with BAS was associated with reduced growth of the intima of the vessel, thereby preventing cardiac allograft vasculopathy during the first year after transplantation (Wang et al. 2015).

The usual dose is 20 mg. It was also studied with a lower dose 10 mg in 17 patients which showed similar efficacy and safety. By the 2-week assessment post-transplant in these patients, there were no deaths, graft failures, or acute cellular rejections for patients ISHLT grade $\geq$ 2R (Kittipibul et al. 2017).

However, there was conflicting evidence of literature where using BAS was associated with increased risk of mortality. Patients from ISHLT registry from 2000 to 2013 were studied and no induction therapy had an improved overall mortality when compared to using BAS adjustment HR = 1.11 (95% CI, 1.04–1.19). BAS also had higher risk of graft failure when compared to no induction therapy (Nozohoor et al. 2020). Where BAS was studied head to head with ATG in pediatric and adult patients, use of basiliximab was associated with all-cause mortality (HR, 1.27; 95% CI, 1.02–1.57; $P = 0.030$) (Ansari et al. 2015a, b).

3 Daclizumab

Daclizumab has shown to reduce the rate of rejection, however, when combined with cytolytic therapy, it had increased mortality (Hershberger et al. 2009; Petrikovits et al. 2005). It was also studied against anti-thymocyte globulin (ATG) for induction therapy and it performed equivalent to ATG in terms of rejection, infection, malignancy (Mullen et al. 2014). When Daclizumab was tested against OKT3, no differences in rejection rate and no subject had severe acute rejection within the first 180 days (Chin et al. 2005; Cuppoletti et al. 2005). Daclizumab when tested against no induction did not result in increased mortality and was beneficial leading to a lower incidence of AR (Kobashigawa et al. 2005). Daclizumab induction therapy reduced the incidence of acute rejection episodes during the first two posttransplant months in HT patients (Petrikovits et al. 2005)

Two doses of Daclizumab were as efficacious as five doses in preventing rejection while maintaining the same benefit of survival in 81 patients (Ortiz et al. 2006).

Some studies have shown that using daclizumab may lead to increased incidence of grade 1 rejections, leading to increased costs (Carlsen et al. 2005). Daclizumab was tested against BAS and no differences were observed in survival or the incidence of rejection (Martin et al. 2015). Interestingly, survival was worse in patients not receiving induction therapy. In 2009, Daclizumab was discontinued from the market.

4 Campath-1 H/Alemtuzumab

Alemtuzumab is a humanized monoclonal antibody against anti-CD52 antibody which acts against mature lymphocytes inducing immunosuppression in patients with heart transplant. It has been studied fairly decently over the last few years. Alemtuzumab was studied against ATG, BAS, and had lower acute rejection rates and lower chances of infection in comparison, however these findings were based off observational studies (Li et al. 2018). When compared to thymoglobulin, the cancer-free survival did not differ in Alemtuzumab (Chivukula et al. 2014). Among 56 patients who were compared between Alemtuzumab and standard induction therapy, grade $\geq$2 rejection at 12 months was not different between alemtuzumab and standard therapy, due to the similar rates between antibody mediated reactions (76.9% vs 96.2%, $P = 0.077$). Rates of cellular reaction were lower as well and alemtuzumab had better renal recovery rates (Gale et al. 2019). This medication also studied in patients with LVADs who develop antibodies, a case series done in three patients who underwent plasmapheresis and were treated with Alemtuzumab allowed for transplantation in these sensitized crossmatch patients (Lick et al. 2008). Induction therapy with Alemtuzumab results in a similar 12-month survival, but a greater freedom from rejection (84.5% versus 51.6%) despite lower calcineurin levels and without the use of steroids (Teuteberg et al. 2010).

5 Rituximab

Rituximab is a chimeric monoclonal antibody against CD20, the main function is to deplete B cells. It prevents the development of new antibodies, rather than removal of the pre-existing antibodies. It was widely used as an immunosuppressant in hematopoietic B cell lymphomas, was also studied as an induction agent in cardiac transplantation. For cardiac patients, it plays crucial role in de-sensitizing patients along with IVIG, plasmapheresis, and bortezomib. In 523 study population, 95 patients had panel reactive antibodies for >10%, of which 21 patients were treated with plasmapheresis, intravenous gamma globulin, and rituximab. This led to the decrease in circulating antibody level from a mean of 70.5 to 30.2% (Kobashigawa et al. 2011). When studied, it associated with reduced vascular reaction by evidence of improvement of LVEF in eight patients (Garrett et al. 2005). It was also associated with improved survival in cardiac allograft AMR in 13 patients as opposed to 20 patients that did not receive it (Ravichandran et al. 2013). Its ability as salvage therapy for refractory AMR was limited to several case

reports identified in the literature (Baran et al. 2004). It is used to treat complications such as lymphoproliferative disorder after cardiac transplant successfully and shown some evidence to treat humoral rejections (Aranda et al. 2002).

On the contrary, in a randomized controlled trial, among the 163 patients who received rituximab 1,000 mg on days 0 and 12 post-transplantation, there was no mean change in atheroma volume indicating that it did not reduce the CAV progression but instead enhanced it leading to accelerated progress during the first year. There were no differences in mortality as noted in the study (Starling et al. 2019). When studied for late AMR in 20 patients, 45% patients received rituximab, patients ended up having poor prognosis, such as early death, recurrence, or persistence of AMR and fulminant CAV (Coutance et al. 2015).

6 CTLA-4-Ig/Belatacept

Belatacept is a high-affinity CTLA4Ig used as prophylaxis for graft rejection in adults receiving a renal transplant, its use was later extended to heart transplantation and is being used as a maintenance regimen in selected cases. It interferes with the binding of the CD28 on T cells & B7 ligands on antigen presenting cells, suppresses the T-lymphocyte co-stimulation leading to immune tolerance. It was widely tested in animal models and some observational, retrospective studies in humans (Chen et al. 2015). Forty patients were started on Belatacept at some point (within 3 months and after) after heart transplant which delays post-operative kidney failure, stabilizes renal function (Launay et al. 2020). Belatacept along with proteasome inhibition therapy in four patients reduced HLA I and II antibodies as well as sustained suppression of donor specific antibodies (Alishetti et al. 2020). It is easier to monitor than calcineurin inhibitor-based regimens (since no therapeutic drug monitoring is required), although it carries a risk of posttransplant lymphoproliferative disorder (PTLD) (Martin et al. 2013). Short-term treatment with CTLA-4 Ig induced modest prolongation of cardiac allograft survival, they prolonged survival >100 days if combined with PMN depleting antibodies (Tarek et al. 2005). Few case reports described where Belatacept was successfully used and it reversed renal failure in a patient with combined heart, liver, and kidney transplant (Kumar et al. 2018). It was used as a maintenance regimen in lone heart transplants as well but the evidence is limited to case reports alone. In a 26-year-old female, Belatacept was initiated on top of tacrolimus and sirolimus, no rejection episodes were detected (Enderby et al. 2014).

7 Calcineurin Inhibitors (Cyclosporine and Tacrolimus)

7.1 Cyclosporine (CSA) and Tacrolimus (TAC)

Cyclosporine inhibits the enzyme calcineurin in T cells, leading to prevention of differentiation of T cells (Söderlund and Rådegran 2015). Tacrolimus acts similarly by suppressing T cell activation, subsequently inhibiting IL2 activation. In a meta-analysis of 885 patients comparing CSA to TAC, there were no statistical differences in one-year mortality rates, however, risk of rejection was lower with TAC at 6 months and 1 year (Ye et al. 2009). Tacrolimus group had a significant increase in the risk of diabetes mellitus. Both groups had equal rates of incidence of malignancy or renal failure requiring dialysis. Cyclosporine also resulted in more weight gain in 101 patients when tested against tacrolimus (López-Vilella et al. 2015).

TAC is superior to CSA when studied for intimal thickening, vascular remodeling, and microvascular endothelial function (Petrakopoulou et al. 2006). Cyclosporine was largely replaced by tacrolimus (TAC) in regards to reduction of side effects such as hypertension, hyperlipidemia, gingival hyperplasia and when microemulsion CSA mortality was compared with tacrolimus, it resulted in lower mortality and less acute severe biopsy-proven rejection in a meta-analysis of randomized controlled trials among 952 patients (Penninga et al. 2010). This study also showed that both CNI are equally nephrotoxic as opposed to the earlier belief that tacrolimus causes less incidence of renal failure. The nephrotoxicity prompted the use of mycophenolate mofetil or mTOR inhibitors in the recent times (Bennett 1996). Some authors have also combined CSA with Everolimus, which maintained the same rejection rates with a reduced dosage of CSA and trough levels and stable kidney function (Schweiger et al. 2006). CSA nephrotoxicity improved after the dose reduction along with MMF when tested in 34 patients. The other arm in the study was a combination of Everolimus with reduced dose CSA, however, the renal benefit was limited to patients without baseline proteinuria (Potena et al. 2012). Of note, reducing the dose of tacrolimus did not prevent or result in superior long-term renal function after 8 years when compared to doing the full dose (Guethoff et al. 2015). In 49 patients, there were no differences in intimal proliferation between CSA and TAC or in the development of CAV in the maintenance treatment with MMF and steroids after 1 year of HT (Sánchez-Lázaro et al. 2010).

In NOCTET (heart and lung) trial, where the study group was Everolimus and reduced dose CNI with standard dosing, it showed that among patients with pre-existing advanced renal failure (n = 194), the study group showed improved renal function. Improvement was limited to patients with <4.6 years transplant time (Arora et al. 2012). SCHEDULE trial studied 115 patients with heart transplant and randomized them into Everolimus with complete cyclosporine withdrawal 7–11 weeks after transplant vs standard immunosuppression. The Everolimus group ($n = 37$) had significantly reduced CAV progression as compared with the cyclosporine group ($n = 39$) at 1 and 3 years (Arora et al. 2015, 2018). Some of these patients included patients with moderate kidney disease. ACR was slightly more

frequent in the study group as compared with the control group. Systolic blood pressure also lowers when compared to the CNI based regimen, most pronounced in the patients allocated to Everolimus (Andreassen et al. 2019).

One of the major side effects as noted above with calcineurin inhibitors are its adverse effects on kidney function. As we know that 10% of heart transplant patients develop ESRD by year 5, leading to a greater mortality risk. In some cases, risks outweigh benefits with CNIs especially with the evolvement of newer immunosuppressants.

8 Proliferation Signal Inhibitors/Mammalian Target of Rapamycin Inhibitors

8.1 Everolimus

Via blockade of mTOR pathway, Everolimus and Sirolimus inhibit proliferation of fibroblasts and smooth muscle cells which play an important role in development of CAV (Jennings et al. 2018). However, only small percentage of HT patients receive mTOR inhibitors due to the high incidence of side effects and acute cellular rejection (Eisen et al. 2013). Everolimus when tested against azathioprine in 634 patients showed that the incidence of vasculopathy was significantly lower in the Everolimus group when compared to azathioprine, lower incidence of cytomegalovirus infections and higher incidence of bacterial infection were higher in the Everolimus group. Serum creatinine was also higher in the Everolimus group (Eisen et al. 2003).

Everolimus with reduced dose CSA was tested against MMF with standard dose CSA. Everolimus was noninferior to MMF when it came to acute rejection, acute rejection associated with hemodynamic compromise, graft loss/re-transplant, death (Eisen et al. 2013). Non-fatal adverse events were more common with Everolimus in that study.

Everolimus was also tested against MMF in 553 patients and IVUS was performed after 1 year and it showed a reduced incidence of CAV in the Everolimus group (Kobashigawa et al. 2013a). An open label study of 176 patients randomized to either receive MMF or Everolimus showed non-inferiority in terms of renal function and had equivalent efficacy at 1 year post-transplant (Lehmkuhl et al. 2009). Everolimus also performed similarly in terms of survival when compared to MMF in randomized controlled trial at 1 and 5 years when treated with CSA and steroid (Wang et al. 2010). Everolimus with low dose CNI followed by CNI free therapy maintained better long-term renal function and significantly reduced CAV when compared to the standard CNI treatment after 5–6 years of the transplant. This was done as a follow-up study after SCHEDULE study (Gustafsson et al. 2020).

In an RCT of 181 patients, patients receiving delayed Everolimus (4–6 weeks) with MMF as a bridge appeared to provide early safety benefit because of lower clinical significant side effects when compared to immediate Everolimus use (<144 h). They both had same efficacy (Potena et al. 2018). Everolimus with low dose CNI followed by CNI free therapy (after 7–11 weeks) with standard dosing in

115 patients tested head to head, the Everolimus group maintained better long-term renal function and significantly reduced CAV (Andreassen et al. 2014).

Fifty-eight patients whose immunosuppressant regimen was converted from CNIs to Everolimus were followed for almost 2 years. Mean creatinine clearance increased in the Everolimus group and mean blood pressure lowered. Adverse effects were more in the Everolimus group, however, they resolved without any intervention (Engelen et al. 2011). CNI based side effects such as peripheral edema, tremor, hirsutism also improved with Everolimus in most patients after CNIs were removed (Stypmann et al. 2011).

In a study of 108 heart transplant patients, CSA plus Everolimus vs CSA plus MMF vs tacrolimus plus MMF, no difference in mortality was noted. The Everolimus plus CSA group showed a significantly less efficacy failure when compared to CSA plus MMF or tacrolimus plus MMF (Wang et al. 2008). Everolimus 1.5 or 3 mg was tested against azathioprine and they were followed for 24 months, Everolimus had a reduced incidence of acute rejection and limited the allograft vasculopathy (Viganò et al. 2007). Another study which looked at various Everolimus doses showed that unlike CSA, increasing Everolimus doses was not related to higher rate of renal dysfunction. Biopsy proven acute rejection was reduced in Everolimus trough levels $\geq$3 ng/mL (Starling et al. 2004).

Everolimus is also associated with lower incidence of CMV infection when compared to azathioprine and MMF (Kobashigawa et al. 2013b; Viganò et al. 2010).

9 Sirolimus

Sirolimus inhibits the mammalian target of rapamycin (mTOR) and reduces the cardiac allograft vasculopathy (CAV). Sirolimus has anti-proliferative and anti-migratory actions. Using sirolimus $\geq$3 years after transplantation, the burden of intravascular ultrasound based CAV was substantially reduced. Treatment with azathioprine or mycophenolate mofetil did not affect the results. Moreover, renal function improved in the sirolimus group (Eugenia et al. 2007). Another study was done by Mancini et al. where sirolimus was tested against the current immunosuppression. In that study, three patients reached primary end-point which was slowed disease progression versus 14 patients in the control group ($P < 0.001$) (Donna et al. 2003). In a randomized controlled study of 57 cardiac transplant patients with mild to moderate renal insufficiency, converting from CNIs to sirolimus improved renal function. This finding was better observed in patients without diabetes (Zuckermann et al. 2014). Four hundred and two heart transplant patients treated either with CNI or conversion from CNI to sirolimus underwent an analysis of the effect of these strategies on the development and progression of cardiac allograft vasculopathy. The progression of plaque volume and plaque index was decreased with sirolimus when compared to CNI. All-cause mortality was lower after a follow-up of 8.9 years along with lower incidence of CAV with sirolimus when compared to CNIs (Rabea et al. 2018). Sirolimus was directly compared with azathioprine in combination with cyclosporine and steroids. There was significant absence of progression of medial

proliferation in patients with sirolimus which continued at 2 years. These findings favored the use of sirolimus as compared to azathioprine (Anne et al. 2004). Sirolimus also inhibits adverse ventricular remodeling, decreased LV mass and LV mass index (Kushwaha et al. 2008).

Sirolimus when converted to from CNIs improved renal function in cardiac transplant patients with renal impairment, however, rates of acute rejection were higher in the Sirolimus group, in a total of 116 patients. Sirolimus was associated with diarrhea, infection, and rash (Zuckermann et al. 2012). In 45 patients who had CNIs removed from their regimen and substituted with Sirolimus, outcomes showed that IVUS demonstrated reduced plaque progression in the sirolimus group, if conversion occurred in <2 years after transplant. Five-year survival was improved with Sirolimus and that group had freedom from cardiac related events (Yan et al. 2012).

Sirolimus also has anti-tumor angiogenesis properties, there has been evidence to reduce skin and non-skin malignancies 5 years after renal transplant when compared to cyclosporine (Campistol et al. 2006; Guba et al. 2002).

Patients with Sirolimus have a high incidence of side effects, around 70% of patients had an incidence of a side effect according to one study over a period of 10 years. They recommended maintaining levels at a lower end (Thibodeau et al. 2013).

10 Bortezomib

Bortezomib is a proteasome inhibitor which has a proapoptotic effect on plasma cells, thereby decreasing antibody production in patients awaiting a heart transplant. Around 20% of patients receiving a heart transplant are sensitized. It was studied in renal transplants more widely than in HT (Everly et al. 2012). Use of this medication decreased the mean panel reactive antibodies from 62% to 35%, just after one dose in six of the seven patients studied (Patel et al. 2011). For 30 patients awaiting a heart transplant according to a study done by Patel et al., one cycle of plasmapheresis and Bortezomib was used to de-sensitize, it reduces the HLA antibody burden in a majority of sensitized patients. If the 22 patients that underwent transplantation, the 1 year survival rate was 100%, freedom from ATR was 73.9% (Patel et al. 2015a). Bortezomib was also used in patients who developed AMR. This in conjunction with IVIG, rituximab, plasmapheresis helped treat AMR successfully (Ludwig et al. 2020). Khuu et al. also described a case series of nine patients with AMR who were given Bortezomib after failing conventional therapies, of which eight patients improved significantly in DSA and/or negative biopsy. Class 1 antibodies responded better than class II, median duration of antibody response was 76 days after 1–2 cycles of Bortezomib. Sixty-day survival in these patients was 100% (Khuu et al. 2015).

11 Eculizumab

Eculizumab is an anti-C5 monoclonal antibody which inhibits C5 cleavage and prevents the formation of C5-9 membrane attack complex and its downstream effects. It was earlier studied in kidney transplant in 26 patients and the incidence of AMR was low in the Eculizumab group when compared to the control group (Stegall et al. 2011). It was tested in the pediatric population more widely than adult population (Thrush et al. 2016). In a pilot study, eculizumab when administered to nine patients in addition to ATG, steroids, MMF, and TAC showed that survival rate at 12 months was 88%, AMR freedom rate was 75%, and ACR 100% (Patel et al. 2015b). Among 20 patients treated with Eculizumab with nine infusions for 2 months post-transplant, survival at 1 year was 90% with no deaths resulting from AMR (Patel et al. 2021).

12 IVIG

Intravenous immunoglobulin (IVIG) is a pooled IgG extracted from the plasma of thousands of donors. It works via different mechanisms to decrease the circulating antibody burden. It inhibits complement deposition, activation of macrophages, and possible neutralization of cytokines (Ballow 2011). IVIG was routinely used as a part of de-sensitization protocol along with rituximab or some other agents. Some authors noticed a dose-dependent response to IVIG (Edwards et al. 2019). IVIG use is associated with decreased mortality in patients with heart transplant especially if associated with hypogammaglobulinemia (Bourassa-Blanchette et al. 2019). Serious adverse effects occur in $<5\%$ of patients. IVIG was better studied in kidneys when compared to the HT. Fifty-five LVAD patients were either treated with IVIG or plasmapheresis, the authors noticed that use of IVIG caused a mean reduction of HLA class 1 antibodies by 33%, almost nearing non-sensitized patients, waiting times for HT were reduced proving that IVIG is a safe and effective modality (John et al. 1999). It is also used to treat AMR however, no RCTs are available.

13 Other

Carfilzomib (CFZ) was used in two patients who were highly sensitized, two rounds of CFZ decreased PRA to 51% in one patient and one had no donor-specific antibodies (Sacha et al. 2014). FTY720 was studied in animal models of heart transplant since it reduced peripheral lymphocytes (Wang et al. 2003).

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Adverse Effects of Immunosuppression: Infections

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1 Introduction

Immunosuppressive therapies are currently indicated for a wide range of diseases. As new agents emerge and indications evolve the landscape grows increasingly complex. Therapies can target pathologic immune system over-activation in rheumatologic or autoimmune disease, or conditioning and graft versus host disease (GVHD) prophylactic regimens may eliminate or inhibit host immune function to improve graft survival and risk of complication in solid organ transplantation (SOT) or hematopoietic stem cell transplantation (HSCT). With immunosuppressive therapy, infections occur. Complex disease states, host factors, and concomitant therapies contribute to a "net state" of immunosuppression that must be considered and may confound perceived increased infection risks in patients receiving treatment (Roberts and Fishman 2020).

Agents that broadly act across the immune system in dose-dependent fashion, such as corticosteroids, non-myeloablative and myeloablative chemotherapy, predispose patients to a host of bacterial, viral, and fungal infections including opportunistic infections such as *Pneumocystis jirovecii pneumonia* (PJP), tuberculosis or hepatitis B reactivation. Risk factors, mechanisms of immunosuppression, and epidemiology among these broad agents are extensively reviewed elsewhere and will not be covered here. Targeted therapies, both biologic and non-biologic, selectively inhibit the immune system and carry specific individual risks which we aim to describe.

A collection of targeted therapies utilized for patients with inflammatory bowel disease (IBD), multiple sclerosis (MS), rheumatologic diseases, HSCT and SOT are presented, but this chapter is by no means comprehensive. New therapies continue to be developed, and current ones are too numerous to be collected here. These patient

populations are preemptively screened and treated (or receive prophylaxis) for opportunistic infections such as tuberculosis, hepatitis B reactivation, cytomegalovirus (CMV), or toxoplasmosis. This may mitigate infectious risks of therapeutic agents, as pathogens are identified and treated before clinical disease develops. Screening and prevention measures (including immunizations and prophylaxis regimens) as suggested by consensus or local guidelines are assumed and may continue to change as new evidence emerges. A table of hepatitis B reactivation risks and general reactivation risks with monitoring and prevention recommendations based on societal and expert guidance is included at the end of this chapter. Data from published trials, expert opinions, and longitudinal safety monitoring regarding infectious risks associated with therapeutic agents are presented and may be incorporated at the bedside with standards of practice for each individual patient.

2 Non-Biologic Disease-Modifying Therapies and Disease-Modifying Antirheumatic Drugs

2.1 Methotrexate

Methotrexate is employed in rheumatologic, oncologic, and inflammatory bowel diseases (IBD) often as a backbone therapy. It inhibits folic acid metabolism and cytokine production, increases extracellular adenosine, induces peripheral T-cell apoptosis, suppresses IL-1β and IL-6, and broadly inhibits leukocyte activity (Gerards et al. 2003; Walling 2006). Myelosuppression commonly follows and predisposes to infection though inconclusively at lower dosages (Cronstein 1996). Infection risk is highest shortly after initiation and sporadic cases of opportunistic infection have been reported (Kaneko et al. 2006). Tuberculosis reactivation may occur but studies showing a definitive link are lacking (Sadovici et al. 2013). Screening is reasonable, particularly in high prevalence areas or with expectation of future therapies (Sadovici et al. 2013). Studies demonstrating increased infection risk are mixed. A large meta-analysis of placebo-controlled trials in rheumatoid arthritis (RA) showed a small but significant increased infection risk not seen in non-RA populations (Ibrahim et al. 2018).

2.2 Aminosalicylates

Sulfasalazine and its component metabolite 5-aminosalicyclic acid (5-ASA) are utilized in IBD. Multiple formulations of 5-ASA are available with differing delivery sites. Mechanisms of action are not clearly defined, but could include inhibition of prostaglandins, cytokines, lymphocyte DNA synthesis, IL-2 production, or lymphocyte adhesion and function (Rousseaux et al. 2005). Specific infectious risks are not defined. Agranulocytosis and leukopenia occur with sulfasalazine and could predispose patients to infection (Jick et al. 1995). This does not extend to 5-ASA

formulations. Scattered cases of hypersensitivity reactions to sulfasalazine with detectable herpes viruses exist; however, these are limited and without causal proof (Komatsuda et al. 2008; Tohyama et al. 1998). One study reported reductions in surgical site infections in rheumatoid arthritis patients on sulfasalazine compared to other therapies which they proposed may be due to bacterial folic acid synthesis inhibition (den Broeder et al. 2007). No definitive evidence of infectious risk to opportunistic infections is established but risks of agranulocytosis and leukopenia should be considered.

2.3 Pyrimidine Synthesis Inhibitors

Pyrimidine synthesis inhibitors include leflunomide, and its active metabolite teriflunomide. They prevent T-cell activation by antigen presenting cells (Zeyda et al. 2005). They are prescribed in rheumatologic disease and MS but have been used in IBD (O'Connor et al. 2011; Prajapati et al. 2003). Randomized control trials for teriflunomide in MS did not show increased rates of infection compared to placebo and reported only 1 case of intestinal tuberculosis (O'Connor et al. 2011; Confavreux et al. 2014). Two cases of CMV were reported, but leflunomide is active against CMV by theoretical viral capsid formation inhibition (Ariza-Heredia et al. 2014). Active tuberculosis occurs in patients receiving leflunomide though is limited to case reports (Grover et al. 2006). Viral hepatitis screening should be performed before starting therapy due to potential drug hepatotoxicity and possible enhancement of hepatitis B replication (Hoppe-Seyler et al. 2012).

2.4 Thiopurines

Use is declining but azathioprine and mercaptopurine are utilized in IBD, cancer, and certain autoimmune processes. They inhibit nucleic acid metabolism which can result in leukopenia early after initiation and at higher dosages, increasing risk of bacterial infection (Present et al. 1989). Cases of CMV infection are reported in patients with IBD, but leukopenia and the disease itself may be significant risk factors (Present et al. 1989; Hookey et al. 2003). Varicella zoster virus (VZV) reactivations may be more common but risk of specific opportunistic infections is unclear (Gupta et al. 2006).

2.5 Sphingosine Analog

Fingolimod, and newer agents siponimod and ozanimod, function as a sphingosine 1-phosphate analogs causing lymphatic sequestration of lymphocytes (Chun and Hartung 2010). Two, 24-month placebo controlled trials in MS did not demonstrate increased infection rates (though over half reported upper respiratory tract infections), but reported increased mucocutaneous herpes simplex (HSV) and

VZV infections in treatment arms (Calabresi et al. 2014; Kappos et al. 2010). Tuberculosis was reported in only one patient in either trial and reported a home exposure; viral hepatitis was not reported (Calabresi et al. 2014). Sporadic cryptococcus infections, including meningoencephalitis, have occurred (Achtnichts et al. 2015). A trial comparing fingolimod to interferon therapy found more cases of herpesvirus infections in the 1.25 mg arm including fatal cases of disseminated primary VZV and HSV encephalitis (Cohen et al. 2010). No cases of HBV reactivation were reported and standardized screening was not performed. At least nine rare cases of progressive multifocal leukoencephalopathy (PML) have occurred including one patient without a history of natalizumab administration (Berger 2017). Additional studies document severe VZV infection with fingolimod solidifying the association (Gross et al. 2012; Ratchford et al. 2012). Trials of siponimod report increased VZV infections (Kappos et al. 2018). Trials of ozanimod, a selective inhibitor of sphingosine 1-phosphate receptor subtypes 1 and 5, compared to interferon β1a did not show increased rates of VZV infection, but patients were screened for VZV serology or VZV vaccination history prior to enrollment (Comi et al. 2019). Varicella zoster serostatus evaluation and vaccination should be performed prior to therapy (Arvin et al. 2015).

2.6 Dimethyl Fumarate

Dimethyl fumarate activates nuclear 1 factor-like 2 enhancing antioxidant response and altering dendritic cell differentiation in MS (Gold et al. 2012). Randomized, placebo-controlled trials report infections in a majority of patients, typically upper respiratory infections (URI) and urinary tract infections (UTI), but rates did not differ among treatment groups (Gold et al. 2012; Fox et al. 2012). No opportunistic infections were reported at 2 years in either trial and latent TB screening was not standardized. Longitudinal infectious risk is unclear, but there are sporadic case reports. PML has been documented, and at least 1 patient only received dimethyl fumarate (Sweetser et al. 2013). One case of severe disseminated VZV with neurologic deficits has occurred (Ma et al. 2016). Dimethyl fumarate has a favorable infection profile compared to placebo, though rare cases of PML warrant further study.

3 Janus Kinase (JAK) Inhibitors

Janus Kinase (JAK) inhibitors include tofacitinib, baricitinib, and upadacitinib. They are utilized in RA and IBD. Most experience is with tofacitinib. JAK inhibitors prevent lymphocyte activation through inhibition of inflammatory cytokines (Meyer et al. 2010). Large, placebo-controlled trials found increased rates of neutropenia and serious infections including cellulitis or abscesses though patients were often screened for HBV, hepatitis C (HCV), and TB (Fleischmann et al. 2012; Kremer et al. 2013; van der Heijde et al. 2013; van Vollenhoven et al. 2012). Trials also

report cases of tuberculosis (Fleischmann et al. 2012; Kremer et al. 2013; van der Heijde et al. 2013; van Vollenhoven et al. 2012). Opportunistic infections reported include PJP, cryptococcus infection, disseminated VZV, CMV sialadenitis, and esophageal candidiasis (van der Heijde et al. 2013; Lee et al. 2014). Longitudinal safety studies confirm an increased risk of VZV infection associated with tofacitinib (Winthrop et al. 2014). A trial of tofacitinib in IBD reported higher infection and VZV rates compared to placebo, highlighting the risk across multiple populations (Sandborn et al. 2017). A longitudinal safety study over eight years revealed no new opportunistic infection risk, but many patients with the potential for HBV reactivation were screened out of clinical trials (Cohen et al. 2017). Case reports of reactivation exist (Chen et al. 2018).

Trials of baricitinib report infections at similar rates compared to placebo (Dougados et al. 2017; Keystone et al. 2015; Tanaka et al. 2016). While two trials reported no opportunistic infections, Dougados et al. reported cases of TB and VZV in baricitinib groups (Dougados et al. 2017; Keystone et al. 2015; Tanaka et al. 2016). Trials for upadacitinib, a more selective JAK-1 inhibitor, continue but this agent appears to carry lower infectious risk compared to methotrexate (Smolen et al. 2019). Herpes zoster infection occurred more often with upadacitinib.

Ruxolitinib selectively inhibits JAK1 and JAK2 and is used to treat chronic GVHD after HSCT and myelofibrosis. Placebo-controlled trials in myelofibrosis reported cases of bacterial infection but differences between arms were not evaluated (Verstovsek et al. 2012). While no opportunistic infections were reported, case reports of cryptococcal infection, HBV reactivation, TB, CMV retinitis, and PML exist, but this may reflect the net state of immunosuppression rather than drug effect (Caocci et al. 2014; Colomba et al. 2012; von Hofsten et al. 2016; Wathes et al. 2013; Wysham et al. 2013). Increased cryptococcal infection risk exists and should be considered in differential diagnosis of fungemia, meningoencephalitis, or pneumonia in this population, but cases remain limited (Harvey et al. 2019). Future longitudinal studies may reveal risks. Vaccination for VZV and screening for opportunistic infections including TB and viral hepatitis should be performed prior to starting JAK inhibitors.

4 Integrin Antibodies and Adhesion-Molecule Inhibitors

4.1 Natalizumab

Selective adhesion-molecule inhibitors are prescribed for MS. Natalizumab functions as an α4β1 integrin antibody (Epstein et al. 2018). An initial randomized, double-blind trial showed no difference in rates of infection between natalizumab and placebo groups (Miller et al. 2003). However, post-marketing studies and real-world experience have reported potential pathogens. Sporadic HSV or VZV cases including meningoencephalitis have been reported, suggesting a temporal relationship to drug therapy but studies demonstrating a link are lacking (Fine et al. 2013). Initial trials did not report latent TB reactivation, but similar integrins are involved in

immune response against pulmonary *Mycobacterium tuberculosis* infection (Polman et al. 2006; Rudick et al. 2006). Longitudinal studies have not found an increased risk of active TB (Mulero et al. 2012). Latent tuberculosis screening prior to natalizumab therapy is reasonable. At least one case of acute liver failure and death from HBV reactivation has been reported in a patient on natalizumab, but major trials did not report cases (Miller et al. 2003; Polman et al. 2006; Rudick et al. 2006; Hillen et al. 2015).

The most well-described infectious complication associated with natalizumab is PML. Studies have shown incidences from 2.13 to 20.7 per 1,000 treated patients (Schwab et al. 2017; Vennegoor et al. 2015). JC virus (JCV), the viral pathogen responsible for PML, seroprevalence stands at 50–90% of the adult population and clinical disease in immunocompetent hosts rarely occurs (Brew et al. 2010). In patients treated with natalizumab, prior immunosuppression, prolonged duration of treatment and JCV specific antibodies are risk factors for PML (Schwab et al. 2017). An expert panel recommends JCV serologic screening at baseline, 12 months after initiation, and every 6 months thereafter (McGuigan et al. 2016). After an anti-JCV antibody index level of 1.5, additional screening is not needed. Imaging, with MRI, should occur annually, with increasing frequency as anti-JCV antibody index increases, as findings may precede clinical disease (McGuigan et al. 2016). Once PML develops, outcomes are poor and neurologic sequelae are common (Brew et al. 2010). No therapies, other than cessation of natalizumab, have demonstrated significant treatment benefit though rare cases utilizing JC virus specific donor lymphocytes have shown potential promise for future study (Berzero et al. 2021). Trials have evaluated natalizumab in Crohn's disease but longitudinal studies are less robust (Ford et al. 2011).

4.2 Vedolizumab

Vedolizumab is a humanized monoclonal α4β7 integrin antibody which selectively inhibits lymphocyte gastrointestinal tract migration and is utilized in the treatment of Crohn's disease and ulcerative colitis (Soler et al. 2009). Clinical trials in IBD reported no increases in infectious complications (Feagan et al. 2013; Parikh et al. 2012; Sandborn et al. 2013). Longitudinal reviews from these and other trials demonstrated a reduced infection rate overall with vedolizumab compared to placebo but higher rates of gastroenteritis (Colombel et al. 2017). Risk factors included prior anti-TNF failure, corticosteroid use, and narcotic analgesics. Tuberculosis was reported at a rate of 0.1 events per 100 patient years and 3 of the 4 recorded cases had negative latent tuberculosis screening testing at initiation. Hepatitis B reactivation was not reported, and although a clear risk has not been demonstrated with vedolizumab therapy, it is considered to carry a moderate risk of reactivation (Loomba and Liang 2017). Patients on vedolizumab have non-inferior immunologic responses to hepatitis B vaccination so it should be administered if indicated (Harrington et al. 2020). Notably 10% of reviewed patients reported unexplained neurologic symptoms but none were diagnosed with PML in a 2-year follow-up

period (Colombel et al. 2017). The authors concluded that at a similar rate of JCV seropositivity, 6–7 cases would be expected if vedolizumab had a similar PML risk as natalizumab.

5 Tumor Necrosis Factor (TNF)-Alpha Inhibitors

TNF-alpha inhibitors led to breakthrough advances in IBD and rheumatologic disease but carry infectious risks. Class drugs include monoclonal antibodies against TNF-alpha (adalimumab, golimumab, and infliximab), pegylated fragment of a humanized anti-TNF-alpha antibody (certolizumab), and soluble TNF-alpha receptor (etancercept). They inhibit neutrophil and macrophage function, granuloma formation and stability, increasing risks for granulomatous and intracellular infections (Harris et al. 2008). Extensive randomized trials show mixed rates of bacterial, fungal, and viral infections, but a large meta-analysis of 106 trials in rheumatologic patients showed increased risks of serious infection with standard dosing (Singh et al. 2015). Infectious risks may be highest early in therapy (Galloway et al. 2011). Higher TB risk exists for all agents, though etanercept may be lower, and screening should be standard (Dixon et al. 2010). Risk of non-tuberculous mycobacterial infections is also increased (Winthrop et al. 2013). Longitudinal studies demonstrate higher rates of granulomatous infection with infliximab than etancercept, and cases of coccidioidomycosis, histoplasmosis, nocardiosis, cryptococcus, listeriosis, and candidiasis along with tuberculosis have been described (Wallis et al. 2004). Endemic mycoses infections occur earlier after therapy initiation and at higher rates compared to alternative agents (Bergstrom et al. 2004). Invasive fungal infection with aspergillus, zygomycetes, and PJP may occur (Wallis et al. 2004; Tsiodras et al. 2008). Most evidence presented covers studies of adalimumab, infliximab, or etancercept, but similar risks likely occur with certolizumab and golimumab for which additional longitudinal investigation is required (Keystone et al. 2009; Smolen et al. 2009).

Herpes zoster infection rates are higher after adalimumab and infliximab use (Strangfeld et al. 2009). Hepatitis B reactivation, including cases of fulminant hepatitis, has been documented (Zingarelli et al. 2009). Antiviral therapy has been utilized successfully while on anti-TNF-alpha therapy. Screening, including surface antigen and both core and surface antibodies, should be performed prior to initiation of therapy. Vaccination or antiviral prophylaxis should be given if indicated and anti-TNF-alpha treatment is needed (Table 1) (Singh et al. 2016; Di Bisceglie et al. 2015). Evidence indicating worsening of hepatitis C in chronic quiescent disease due to treatment is lacking, but screening should be performed. The American College of Rheumatology recommends etancercept as the drug of choice in patients with active hepatitis C if needed (Singh et al. 2016).

Infliximab may be also used for steroid-refractory GVHD after HSCT. While these patients have increased risks of fungal infection or CMV disease, infliximab carries an additional risk compared to other agents, particularly for invasive fungal infection and some experts recommend mold prophylaxis if infliximab is needed

(Couriel et al. 2004). Screening for latent mycobacterial infection, viral hepatitis, and fungal infections in high-risk areas is recommended and should be performed before initiation of anti-TNF-alpha therapy. Active infection with these or other bacterial, viral, or fungal infections may preclude use. Prophylaxis could be considered with recurrent herpesvirus infection, chronic hepatitis B infection, or molds colonization and infection in HSCT patients.

6 T-Cell Costimulatory Blockers

6.1 Abatacept

Abatacept, the first T-cell costimulatory blocking agent developed, is a CTLA-4 IgG1 fusion protein which blocks CD28 binding and disrupts T-cell activation (Judge et al. 1996). Abatacept may be used to treat RA, psoriatic arthritis, and juvenile idiopathic arthritis. Infectious complications of abatacept are rare but bronchopulmonary infections have been most commonly observed. A 2009 meta-analysis found no differences in infection rates when comparing patients receiving abatacept to those receiving placebo (Salliot et al. 2009). Further, when compared to patients receiving TNF-alpha inhibitors and rituximab, patients receiving abatacept had a significantly lower risk of infections requiring hospitalization (Yun et al. 2016).

6.2 Belatacept

Belatacept, a daughter protein of abatacept and more potent T-cell inhibitor, selectively blocks costimulatory pathway for T-cell activation and is used as de novo or conversion from calcineurin inhibitor (CNI) maintenance immunosuppression after kidney transplantation (Perez et al. 2018). Data in liver and thoracic transplantation is limited though emerging. In the BENEFIT and BENEFIT-EXT trials urinary tract and CMV infections were most common but no differences were seen between intensive belatacept, less intensive belatacept, and cyclosporine treatment groups (Durrbach et al. 2016). Additionally, there were no differences in rates of serious infections between groups. A large, single-center, retrospective study found significantly higher rates of low level CMV viremia when belatacept was used without tacrolimus (Adams et al. 2017). This increased CMV rate was thought to be due to higher rates of rejection in this group which was treated with thymoglobulin and steroids. More recently, a single-center retrospective study of CMV seronegative kidney transplant recipients found a higher incidence of CMV viremia, higher rates of first-line antiviral failure, and longer time to virus clearance in CMV high-risk patients treated with de novo belatacept-based maintenance regimens when compared to those treated with tacrolimus (Karadkhele et al. 2021).

Early belatacept studies found increased rates of post-transplant lymphoproliferative disease (PTLD) in recipients who were initially Epstein Barr

virus (EBV)-seronegative resulting in an FDA boxed warning for belatacept use in these patients (Grinyo et al. 2010). Though belatacept should be avoided in EBV-seronegative recipients, a 2014 Cochrane systematic review found PTLD risk was similar in recipients receiving belatacept when compared to those receiving calcineurin inhibitors (CNI (Masson et al. 2014). Further, no differences in PTLD risk were seen between EBV seropositive and seronegative groups or between patients receiving high- or low-dose belatacept.

7 Selective B-Cell Depletion and Inhibition

7.1 Anti-CD 20 Monoclonal Antibodies

7.1.1 Rituximab

Rituximab, an anti-CD20-directed monoclonal antibody causes rapid depletion of B cells, interferes with B- and T-cell interactions, can lead to hypogammaglobulinemia, and may have prolonged immune effects lasting 6–-12 months or longer (Thiel et al. 2017). Hypogammaglobulinemia may predispose patients to recurrent sinopulmonary infections and may also require routine administration of intravenous immunoglobulin (Barmettler et al. 2018; Casulo et al. 2013). Late onset neutropenia has also been described and can occur an average 5 months after drug cessation and up to nearly 1 year and in certain high-risk patients, antibacterial prophylaxis has been used during prolonged periods of neutropenia (Breuer et al. 2014). Rituximab can be used for a range of B-cell malignancies, immune disorders such as refractory RA, and for desensitization of highly sensitized or ABO-incompatible transplant recipients, as well as antibody-mediated rejection. Fatal cases of HBV reactivation led to an FDA boxed warning for rituximab use in patients with HBV infection (Martin et al. 2014). All patients should be screened for HBV prior to initiation of rituximab and all other anti-CD20 agents and AASLD guidance for screening is described in Table 1. These agents should be discontinued in patients with HBV reactivations.

The varied and composite immune defects induced by rituximab use increases infectious risk in certain patients. Though trials of rituximab for RA have not demonstrated increased infectious risks, overall it is difficult to assess and estimate specific risks given the broad range of infectious risk in published studies, limited controlled trials, and heterogeneity of concomitant immunosuppressive agents (Grim et al. 2007; Kamar et al. 2010; Kelesidis et al. 2011; Shi et al. 2019). Other opportunistic infections such as PML caused by JC virus reactivation have been described and newer anti-CD20 agents may also increase risk (Focosi et al. 2019; Molloy and Calabrese 2012). Additionally, rates of PJP infection may be higher in patients receiving rituximab when compared to those on TNF-alpha inhibitors (Rutherford et al. 2018).

7.1.2 Obinutuzumab, Ofatumumab, Ocrelizumab

Obinutuzumab, a newer anti-CD20 monoclonal antibody with high in vitro potency, used for CLL and follicular lymphoma can increase the risk for severe respiratory tract infections and VZV reactivations, and invasive fungal infections after monotherapy have been reported (Mikulska et al. 2018; Tse et al. 2015). In a study of the anti-CD20 agent, ofatumumab, used for refractory B-cell CLL, half of the patients developed mild to moderate infections (Coiffier et al. 2008). Additionally, in a trial of ofatumumab for relapsing MS, the most common infections reported were upper respiratory tract infections (39%) and urinary tract infections (10%) (KESIMPTA 2020). Similarly, in studies of ocrelizumab, used for relapsing or primary progressive MS, patients have experienced upper and lower respiratory tract infections, skin infections, as well as HSV and VZV reactivations though serious infections are uncommon (Hauser et al. 2020; Montalban et al. 2017). PML after ocrelizumab monotherapy is rare and limited to case reports (Focosi et al. 2019).

7.2 Other Anti-B-Cell Agents

Inotuzumab ozogamicin, an anti-CD22 antibody-drug conjugate, binds to CD22 resulting in internalization and release of ozogamicin which leads to apoptosis. In an open-label phase 3 trial of inotuzumab ozogamicin vs standard intensive chemotherapy for relapsed or refractory B-cell ALL, febrile neutropenia was more common in the inotuzumab ozogamicin group but the incidence of sepsis and pneumonia was similar between groups (Kantarjian et al. 2016). Finally, belimumab is a monoclonal antibody blocking B-lymphocyte stimulator (BlyS) used for SLE. In a long-term safety study cellulitis and pneumonia were found to be the most common infectious complication (Merrill et al. 2012). Serious infections of the urinary tract, CMV, and PML have been reported (Merrill et al. 2012; Raisch et al. 2016).

7.3 Lymphocyte Depleting Agents

7.3.1 Alemtuzumab

Alemtuzumab is an anti-CD52 monoclonal antibody which causes profound and prolonged (up to 1 year) T- and B-cell depletion as well as neutropenia (Hillmen et al. 2007). It is used to treat MS, CLL, Hodgkin's and non-Hodgkin's lymphomas and is used after SOT to prevent (induction therapy) and treat graft rejection, and after alloHSCT to prevent and treat GVHD (Hillmen et al. 2007; Skoetz et al. 2012; Watson et al. 2005). Increased rates of CMV, HSV, and VZV have been seen in patients treated for NHL and MS and herpetic antiviral prophylaxis is typically used in HSCT and SOT recipients treated with alemtuzumab. During periods of alemtuzumab induced profound CD4 depletion, PJP risk is increased and anti-PJP prophylaxis is recommended. Interestingly, though infectious risk is perceived to be significant, and studies have reported this increased risk, similar rates of infections

have been seen when alemtuzumab has been compared to other induction regimens after kidney transplantation (Morgan et al. 2012). Alternatively, a single-center study found increased rates of opportunistic infections when alemtuzumab was used as rejection therapy when compared to induction therapy (Peleg et al. 2007).

7.3.2 Antithymocyte Globulin

Antithymocyte globulin (ATG) is a polyclonal immunoglobulin that depletes peripheral blood T-cells, B cells with immune effects persisting beyond 1 year. ATG is used to prevent (induction therapy) and treat graft rejection after SOT, certain hematologic disorders, and prevent and treat GVHD in allogeneic HSCT recipients. In the setting of long-lasting lymphopenia, herpesvirus infections, CMV, EBV, and EBV driven PTLD, BK virus, and PJP infections have been described (Arai et al. 2017; Charpentier et al. 2003; Issa and Fishman 2009). After SOT, CMV is common in the setting of ATG use without CMV antiviral prophylaxis (von Muller et al. 2006). An early trial of ATG compared to basiliximab found higher rates of UTIs and non-CMV herpesvirus infections but lower rates of CMV disease (Brennan et al. 2006). A recent meta-analysis found no difference in 1-year infection rate between patients receiving basiliximab when compared to ATG (Wang et al. 2018). Further, a recent single-center study of elderly patients found the use of ATG increased rates of infectious complications (UTIs and CMV) when compared to basiliximab (Pham et al. 2020). The infectious risk of ATG appears to be dose dependent (Issa and Fishman 2009; Kang et al. 2021).

7.3.3 Brentuximab Vedotin

Brentuximab vedotin is an anti-CD30 monoclonal antibody-drug conjugate that causes apoptosis by disrupting the microtubule network and is used for the treatment of relapsed and refractory Hodgkin lymphoma and anaplastic large T-cell lymphoma. Studies in patients after HSCT have provided prophylaxis for herpes viruses and PJP (Moskowitz et al. 2015). Though rare, PML after brentuximab vedotin use has been described (Carson et al. 2014). CMV reactivations, PJP, aspergillus, and pseudomonal pneumonias have been observed in patients receiving brentuximab (Gopal et al. 2012).

8 Interleukin Inhibitors

8.1 IL-1 Inhibitors

Interleukin-1 inhibitors include anakinra, canakinumab, and rilonacept. Clinical use is limited mainly to rheumatologic disease and cyclic fever syndromes. Downstream effects of IL-1 involve both innate and adaptive immunity (Mantovani et al. 2019).

Anakinra is a recombinant human IL-1 receptor antagonist with the most longitudinal data. Large, randomized, controlled trials report either a trend toward increased infections or no difference between groups without notable increased risk of opportunistic infections (Cohen et al. 2004; Fleischmann et al. 2003). It

should be noted that patients in both treatment and control arms received corticosteroids and other DMARDs. While sinusitis and URIs were most common, Fleischmann et al. noted that 74% of patients developing serious infection were able to resume anakinra without additional problem (Fleischmann et al. 2003). An open-label follow-up of several of these patients did find one case each of atypical mycobacterial infection, histoplasmosis and candida esophagitis though two of the patients were on prednisone and/or methotrexate (Fleischmann et al. 2006). Subsequent meta-analyses have suggested this increased infection risk may only be at higher doses and also do not report cases of opportunistic infection (Salliot et al. 2009).

Rilonacept is a human dimeric fusion protein composed of an extracellular component of IL-1 receptor and the Fc portion of IgG1 which binds IL-1 subunits to inhibit activity. Higher rates of infection were shown in one early study but not found in another (Hoffman et al. 2008). Opportunistic infection was not reported in this study, but at least one case of *Mycobacterium avium* complex was documented and screening for tuberculosis is still recommended (Koo et al. 2011; Salvana and Salata 2009). Studies of canakinumab, an IL-1β antibody, have shown higher rates of infection compared to placebo but no difference in rates of tuberculosis or reported cases of other opportunistic infections (De Benedetti et al. 2018; Ridker et al. 2017). Infection rates with IL-1 inhibitors may be increased overall predominantly with respiratory tract infections, but studies demonstrating increased risks of opportunistic infection are lacking.

8.2 IL-2 Inhibitors

Basiliximab is the primary humanized IL-2 receptor antibody utilized in clinical practice. Daclizumab was previously used, but withdrawn from the market for safety concerns, though much of the known literature is from patients receiving daclizumab. IL-2 inhibitors have been employed as conditioning regimens in SOT recipients including heart, liver, and kidney allografts and may reduce need for calcineurin inhibitors, steroids or serve as an alternative to antithymocyte globulin (Brennan et al. 2006; Ansari et al. 2015; Emre et al. 2001; Liu et al. 2004). Some studies have found no increased rates of death due to infection with IL-2 inhibitor use but did not report specific episodes (Morris et al. 2005). A Cochrane review did find IL-2 inhibitor receptor inhibitor treated patients had a trend toward less CMV infection at 3 and 6 months which reached statistical significance at 12 months but this is confounded by prophylaxis versus preemptive strategies (Webster et al. 2010). Additionally no difference was noted in CMV infection rate compared to other biologics including muromonab-CD3 or alemtuzumab. In a study of liver transplant patients receiving basiliximab, overall reported infections were lower compared to patients receiving steroids and no opportunistic infections were reported (Liu et al. 2004). Most patients had chronic hepatitis B and received lamivudine. A study of renal transplant patients at least 65 years old demonstrated a decreased incidence of bacterial infection, the majority of which were urinary tract infection, and CMV

infection in patients receiving basiliximab compared to thymoglobulin (Pham et al. 2020). One fungal infection was reported, but not further described. In a placebo controlled trial of cardiac transplant patients all receiving cyclosporine, prednisone, and MMF, patients receiving daclizumab had no significant difference in infections reported though there was one case of cryptococcal meningitis in the daclizumab group (Hershberger et al. 2005). Basiliximab has also been used for steroid-refractory GVHD after allogeneic HSCT with some reported cases of bacterial and fungal infections in addition to herpes virus reactivation, though these are not controlled studies and may be due to cumulative and concomitant immunosuppression rather than basiliximab therapy (Massenkeil et al. 2002; Tang et al. 2020). IL-2 inhibitors have been utilized in sporadic studies for IBD with mixed results and further studies are ongoing (Creed et al. 2003; Sands et al. 2012). There do not seem to be specific infectious complications in patients related to basiliximab therapy beyond those seen with other conditioning or treatment regimens.

8.3 IL-6 Inhibitors

The primary IL-6 inhibitors commercially available are tocilizumab, sarilumab, siltuximab, and satralizumab. Most studies include patients receiving tocilizumab. Fewer cases of infection may occur in patients receiving siltuximab, but only tocilizumab will be covered here due to published studies of clinical experience (van Rhee et al. 2015). Whether similar infection risks exist with such agents requires further investigation.

8.4 Tocilizumab

Tocilizumab, a humanized IL-6 receptor antibody, has been used as primary or adjunctive therapy for a variety of rheumatologic disorders, steroid-refractory GVHD after allogeneic HSCT, chronic antibody-mediation allograft rejection in SOT recipients, and the COVID-19 pandemic (Burmester et al. 2014; Choi et al. 2017; Drobyski et al. 2011; Pettit et al. 2021). In patients with Crohn's disease it has shown improved clinical response in patients with refractory disease though there was a non-statistically significant increase in rates of gastrointestinal abscesses or infections in the treatment arms (Danese et al. 2019; Ito et al. 2004). Initial studies of patients with RA treated with tocilizumab showed an increased risk of non-serious infections, mainly bacterial skin and subcutaneous infections or bacterial or viral respiratory tract infections, without an increased risk of hepatitis or tuberculosis when compared to other medications (Campbell et al. 2011). The higher end of published studies report rates of serious infections around 9.1 per 100 patient years with pneumonia occurring most frequently (Sakai et al. 2015). Rates of infection may exceed those found in patients receiving placebo treatment but may not exceed those receiving TNF-alpha inhibitors in statistical analysis (Sakai et al. 2015; Iannone et al. 2018). While some trials have reported no increased rates of

opportunistic infections or tuberculosis, a large meta-analysis included cases of tuberculosis, candidiasis, atypical mycobacterial infection, cryptococcal disease, and PJP (Emery et al. 2008; Schiff et al. 2011). Most clinical studies describe tocilizumab use in the setting of rheumatologic disease. In a series of kidney allograft recipients, there were fewer infections in patients receiving tocilizumab compared with IVIG and rituximab though two cases of PJP were reported in patients receiving tocilizumab (Sethi et al. 2021). During the one year follow-up period after completion of therapy, infections occurred at a rate of 46.3 per 100 patient-years and included cases of CMV, BK virus, VZV, and histoplasmosis. Urinary tract infections were most common and most patients received concomitant immunosuppression including agents such as tacrolimus or mycophenolate. Case series of viral hepatitis in patients treated with tocilizumab including HBV reactivation, hepatitis C, acute hepatitis E, CMV and EBV have been documented (Biehl et al. 2021).

8.5 IL-12/23 Inhibitors

Through binding of the p40 subunit common to both interleukins, ustekinumab inhibits both IL-12 and IL-23 and has been utilized in rheumatologic and inflammatory bowel diseases (Kavanaugh et al. 2014; Sandborn et al. 2012). An early trial in Crohn's disease did not report a difference in rates of infection though patients received other immunosuppressants as well (Kavanaugh et al. 2014). In one trial for psoriasis and psoriatic arthritis overall infection rates, mostly URIs, may have been increased at higher dosing but not maintenance dosing and at least one serious cutaneous VZV infection was reported (Leonardi et al. 2008). No cases of active tuberculosis were reported, but some patients were diagnosed with latent tuberculosis prior to trial entry and received isoniazid. Other subsequent trials did not demonstrate this increased infection rate at the 90 mg dosage and did not report opportunistic infections (Kavanaugh et al. 2014; Papp et al. 2008). A study in ulcerative colitis patients found similar infectious complications with the exception of one patient with CMV colitis and one with legionella pneumonia. Similar to other studies, patients received additional immunosuppressive agents (Sands et al. 2019). Ustekinumab demonstrated a non-significant trend toward lower rates of surgical site infection compared to TNF-alpha inhibitors in Crohn's disease patients (Lightner et al. 2018). Overall when used for psoriatic arthritis ustekinumab does not carry an increased risk of infection compared to other therapies (Kalb et al. 2015).

9 Complement Inhibitor

Eculizumab is a monoclonal antibody that inhibits terminal complement activation by binding to complement factor 5 and is used for atypical hemolytic uremic syndrome-associated thrombotic microangiopathy after kidney transplantation, paroxysmal nocturnal hemoglobinuria, refractory myasthenia gravis, neuromyelitis optica. Terminal complement inhibition increases the risk of infections with

encapsulated bacteria. Most notably, *Neisseria* spp. infection risk is significantly increased and vaccination and antimicrobial prophylaxis targeting *N. meningitidis* are recommended (Winthrop et al. 2018).

10 Calcineurin Inhibitors

Cyclosporine and tacrolimus potently prohibit T-cell activation and proliferation by inhibiting calcineurin, blocking transcription of early cytokine genes. Though these agents are most commonly used to prevent organ rejection after SOT and GVHD after HSCT they have also been used to treat a variety of refractory autoimmune diseases. Infections with CMV, EBV (and EBV driven PTLD), BK virus, and invasive fungal infections have been described with CNI use (Singh 2005). A recent trial found reduced-dose tacrolimus and everolimus was associated with a lower incidence of CMV infection and disease compared to standard dose tacrolimus and mycophenolate (Tedesco-Silva et al. 2015).

11 Mammalian Target of Rapamycin (mTOR) Inhibitors

Sirolimus and everolimus block B- and T-cell activation by impairing pro-inflammatory cytokine responsiveness and also reduce neutrophil migration. Sirolimus and everolimus are used to prevent organ rejection after SOT and everolimus is approved for the treatment of advanced renal cell carcinoma, breast carcinoma, neuroendocrine tumors, and tuberous sclerosis-associated tumors (Qi et al. 2013). Everolimus use in cancer patients has been associated with increased risk of pneumonia and sepsis and a meta-analysis found higher rates of infection-related deaths when sirolimus was used after kidney transplantation (Qi et al. 2013; Schena et al. 2009). Reactivations of TB, VZV, and HBV have been described in cancer patients and infectious screening should be implemented prior to mTOR inhibitor use (Knoll et al. 2014). Interestingly mTOR inhibitors directly inhibit viral replication. Compared to CNI-based regimen, mTOR inhibitor-based regimen may carry a lower risk of CMV infection after kidney transplant and inhibit BK virus replication (Hirsch et al. 2016; Mallat et al. 2017).

12 Mycophenolic Acids

Mycophenolate mofetil (MMF) is utilized for immunosuppression after SOT or HSCT and for various rheumatologic diseases. It decreases early acute rejection rates in SOT and reduces rates of GVHD (Jorge et al. 2008; Vogelsang and Arai 2001). Through depletion of deoxyguanosine triphosphate or induction of T-cell apoptosis it suppresses B and T lymphocytes (Allison and Eugui 2005). In renal transplants, higher infection rates were observed compared to alternative agents (Pourfarziani et al. 2007). MMF may selectively inhibit pathogens such as

hepatitis C, HSV, HIV, influenza or PJP, but it carries increased risks for BK virus or CMV (Ritter and Pirofski 2009).

In SOT, BK viremia, viuria, and nephropathy occur more frequently with MMF (Mengel et al. 2003; Shi et al. 2007). A dose-dependent correlation between MMF and CMV disease has been shown in kidney transplant patients (Moreso et al. 1998). This occurs at higher doses >3 g/day, and studies report no increase in CMV rates at lower dosage (Ritter and Pirofski 2009). Conversely, some studies have shown increased rates of CMV disease even at 2 g/day dosing (Basic-Jukic et al. 2005). Variable and unreported CMV prophylactic or preemptive management strategies in these studies complicate findings. Studies of MMF in heart, liver, and lung transplant have not demonstrated similar findings (Jain et al. 1998; Palmer et al. 2001). Increased CMV antigenemia occurs in HSCT, but studies demonstrating increased CMV disease are lacking (Hambach et al. 2002). Clinicians should be aware of potential BKV and CMV risk of patients on MMF therapy.

Table 1 Risk of Hepatitis B reactivation, monitoring, and prevention (Di Bisceglie et al. 2015)

Immunosuppression	HBV status[a]	Risk	Monitoring[b]	Antiviral prevention/ therapy[c]
Anti-CD20 or HSCT	sAg +	Very high	Baseline HBV DNA	Yes
	sAg −/ cAb +	Moderate		
High-dose corticosteroids (≥prednisone 20 mg equivalent) Cytokine inhibitors	sAg +	High	Baseline HBV DNA	Yes
	sAg −/ cAb +		HBV DNA monitoring every 1–3 months	If HBV DNA becomes detectable
Cytotoxic chemotherapy Anti-TNF-alpha Anti-rejection therapy (SOT) Anti-integrin JAK inhibitors Interleukin inhibitors	sAg +	Moderate	Baseline HBV DNA	Yes
	sAg −/ cAb +		HBV DNA monitoring every 1–3 months	If HBV DNA becomes detectable
Methotrexate Aminosalicylates Thiopurines Pyrimidine synthesis inhibitors Sphingosine analogs Dimethyl fumarate Mycophenolic acids	sAg +	Low	Baseline HBV DNA	Yes
	sAg −/ cAb +		HBV DNA monitoring every 1–3 months	If HBV DNA becomes detectable

[a]All patients should be screened for HBsAg and HBcAb prior to initiation of immunosuppression
[b]If HBV testing is positive expert consultation (Infectious Diseases or Hepatology) is recommended
[c]Nucleos(t)ide analogs entecavir, tenofovir disoproxil fumarate, tenofovir alafenamide may be used (Buti et al. 2017). Lamivudine may be used when entecavir and tenofovir are not available (Huang et al. 2014; Loomba et al. 2008)

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Malignancy: An Adverse Effect of Immunosuppression

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Abstract

Benefits of solid organ transplantation in end stage organ diseases are indisputable. Malignancy is a feared complication of solid organ transplantation and is a leading cause of mortality in patients with organ transplantation. Iatrogenic immunosuppression to prevent graft rejection plays a crucial role in the cancer development in solid organ transplant recipients. Chronic exposure to immunosuppression increases the malignancy burden through deregulation of host immune defense mechanisms and unchecked proliferation of oncogenic viruses and malignancies associated with these viruses. Vigorous screening of candidates undergoing transplant evaluation for malignancies, careful assessment of donors, and vigilant monitoring of transplant recipients are necessary to prevent, detect, and manage this life-threatening complication.

Keywords

Immunosuppression · Malignancy · Solid organ transplant

1 Introduction

Over the past decades, life expectancy of solid organ transplant recipients (SOTRs) has improved significantly due to the tremendous progress made in surgical techniques, immunology, and refinement of medical management including modern immunosuppression. Short-term outcomes of graft and patient survival have changed notably with improved screening for rejection and advances in histopathology. There has not been a significant change in the long-term survival of SOTRs over the past decade, predominantly due to mortality from cardiovascular diseases, infection, and malignancy (Lamb et al. 2011; Meier-Kriesche et al. 2004; Rana et al. 2019). Mortality of SOTRs secondary to infection and cardiovascular diseases improved after implementation of antimicrobial prophylaxis and identification and screening for cardiovascular risk factors along with optimal management of modifiable risk factors (Pilmore et al. 2010). However, malignancy in transplant recipients remains a great challenge and engenders increased mortality burden, reduced quality of life and survival of SOTRs. Risk of cancer is substantially higher in organ transplant recipients compared to the general population. This increased risk is attributed to long-term exposure to immunosuppression and impaired immune surveillance mechanisms associated with chronic immunosuppression. Immunocompromised patients are a heterogeneous group of patients including SOTRs, patients with human immunodeficiency virus (HIV)/acquired immunodeficiency syndrome (AIDS), congenital immunodeficiencies, patients on dialysis and patients receiving chemotherapy and/or radiation (Gatti and Good 1971; Grulich et al. 2007; Maisonneuve et al. 1999; Serraino et al. 2007). These groups of patients share a similar cancer risk profile elucidating a broader understanding of the role of immunosuppression in carcinogenesis (Gatti and Good 1971; Grulich et al. 2007;

Maisonneuve et al. 1999; Serraino et al. 2007). In this chapter, we summarize the adverse effects of immunosuppression and its association with malignancies with a focus on SOTRs who are exposed to chronic pharmacologic immunosuppression to prevent graft rejection.

2 Epidemiology of Malignancy in Immunocompromised Patients

Increased risk of malignant neoplasms in primary immunodeficiency syndromes has been described in the literature decades ago suggesting the role of the immune system in oncogenesis. In kidney transplant recipients (KTRs), the risk of de novo malignancy reverts to pre-transplant level after graft failure suggesting the role of immunosuppression in malignancy and need for adjustment of immunosuppression to reduce those risks (KDIGO 2009; Vajdic et al. 2006; Van Leeuwen et al. 2010). The risk of malignancy in SOTRs is comparable to patients infected with HIV/AIDS after excluding Kaposi sarcoma (KS) and non-Hodgkin lymphoma (NHL) inferring an association between immunodeficiency and carcinogenesis (Grulich et al. 2007; Serraino et al. 2007). Although increased susceptibility for cancers is noted in SOTRs and patients with HIV/AIDS, SOTRs are more prone to develop colorectal cancers, thyroid and lip cancers as opposed to HIV infected populations (Grulich et al. 2007).

Solid organ transplantation (SOT) has been recognized as the gold standard treatment option for patients with end stage organ failure and survival advantage outweighs the adverse effects of immunosuppression in these patients (Rana et al. 2015). Choice of immunosuppressive regimens in SOTRs requires careful consideration of various factors such as risk of rejection, infection, and malignancy. There is a reported two-to-four-fold greater risk of malignancy in SOTRs compared to the general population matched for age, gender, and race. The magnitude of malignancy risk is variable based on type of malignancy and the transplanted organ. Nonetheless, the increased risk is persistent in all SOTRs regardless of the transplanted organ and despite the exclusion of patients with preexisting neoplasms prior to organ transplantation (Acuna et al. 2016; Chapman et al. 2013; Collett et al. 2010; Engels et al. 2011). Analysis of a large cohort of SOTRs derived from linking between population-based transplant and cancer registries in the United States (US) between the years of 1987 and 2008 demonstrated a clear increased risk of malignancies in SOTRs. Data was analyzed in 175, 732 SOTRs with a cohort comprised of 58.4% KTRs, 21.6% liver transplant recipients, 10% heart transplant recipients and 4% of lung transplant recipients. The incidence of malignancy per 100,000 person years was 1,375 with an excess absolute risk (EAR) of 719.3 per 100,000 person years and a standardized incidence ratio (SIR) of 2.10 [95% CI, 2.06-2.14] suggestive of an exaggerated risk compared to the general population. The increased burden is noted for both cancers of infectious and noninfectious etiology (Engels et al. 2011).

Non-melanoma skin cancer (NMSC) and lip cancers, post-transplant lymphoproliferative diseases (PTLD), and KS and anogenital cancers are frequently seen in SOTRs. In addition, an elevated risk for other cancers such as urogenital cancers, cancers of kidney and thyroid gland have been reported in organ transplant recipients (Acuna et al. 2016; Agraharkar et al. 2004; Buell et al. 2005; Chapman et al. 2013; Collett et al. 2010; Engels et al. 2011; Wimmer et al. 2007). Life expectancy of SOTRs is expected to be compromised by malignancy rather than cardiovascular events in the upcoming years due to the improvements in management of cardiovascular risk factors. Furthermore, cancers in SOTRs are biologically more aggressive and are associated with worse outcomes (Ajithkumar et al. 2007; Campistol et al. 2012; Hall et al. 2013; Vegso et al. 2007).

3 Pathogenesis of Malignancy in Solid Organ Transplant Recipients

Carcinogenesis in SOTRS is a result of complex and dynamic interplay of multiple factors including genetic, host, and environmental factors. Increased incidence of cancers in SOTRs is driven by altered dynamics of host immune surveillance, oncogenic viral infections, and direct carcinogenic effects of immunosuppressive agents (Ajithkumar et al. 2007; Campistol et al. 2012; Hall et al. 2013; Vegso et al. 2007). In addition, underlying chronic disease that prompted the transplantation and associated risk factors may predispose SOTRs to higher risk of malignancies. Natural immune defense mechanisms minimize the risk of neoplastic transformation by elimination of tumor cells, prevention of inflammation, and cell protection. Dysregulation of immune systems by pharmacologic immunosuppression promotes neoplastic transformation and growth by altering mechanisms of early detection and eradication of subclinical tumor cells, immune evasion of tumor cells, bolsters inflammation and proliferation of oncogenic viruses (Sherston et al. 2014). Tumorigenesis is a multistep process and is characterized by sustained proliferative signaling, insensitivity to growth suppressor signals, neo-angiogenesis, evasion of immune mediated destruction, resistance to apoptosis, invasion of tissue and metastasis and metabolic rewiring (Fouad and Aanei 2017; Hanahan and Weinberg 2011). Carcinogenesis in transplant is dominated by impaired immune modulation including reduced ability to eradicate the tumor cells, escape and evasion of neoplastic cells fostering a “tumor microenvironment” (Fouad and Aanei 2017; Hanahan and Weinberg 2011).

3.1 Immune Surveillance

Immune surveillance is an essential host defense mechanism against the development of cancers and for the maintenance of cellular homeostasis. Paul Ehrlich introduced the concept of repression of neoplastic cells by the host immune system in 1909 which generated impassioned debate for the decades to follow. He proposed

that a normally functioning host immune system destroys the subclinical tumor in its latency prior to clinical manifestation. The theory of "immunological surveillance of neoplasia" was reappraised by Lewis Thomas and Sir Frank Macfarlane Burnet in the late 1950s. Thymus dependent immunologic response was theorized to offer defense against tumor development by early detection and elimination at incipient stage. The central theme of this theory was that an immunocompetent host would be less susceptible to cancer development compared to an immunodeficient host. However, mice experiments by Carlos Martinez demonstrated reduced incidence of mammary tumors in mice that had undergone thymectomy compared to the group with intact thymus (Burnet 1970; Martinez 1964). Prospective role of immunosurveillance in carcinogenesis was summarized by Keast based on high incidence of tumors during extremes of age when immune system is nascent or senescent, with use of immunosuppressant medications, after thymectomy in animal experiments and in patients with disorders of cell mediated immunity. The association between immunological disorders and development of reticuloendothelial cancer without implying any causal effect was presented by Doll et al. (Doll and Kinlen 1970). The theory of "immunological surveillance" encountered strong criticism following the nude mice experiments by Stutman et al. and argued against carcinogenic potential of immunosuppressed state (Stutman 1979). Nonetheless, the advances in immunobiology and mice genetics rekindled the interest in the mystic role of the immune system in recognizing and destroying the tumorigenic cells. Pioneering work of Shakaran et al. validated the paradoxical role of the immune system in carcinogenesis and engendered the conceptualization of immunoediting. Over the past two decades, the theory of immunosurveillance evolved into a broader and more widely accepted concept of immunoediting that addresses not only the prevention of tumors, but also the immunogenicity of tumor cells. Immunoediting is a dynamic process characterized by three phases including elimination by immunosurveillance, equilibrium, and immune evasion leading to the escape phase (Shankaran et al. 2001). Chronic pharmacologic immunosuppression in SOTR leads to uninterrupted proliferation of tumor cells due to reduced threshold of surveillance leading to escape from immune elimination. Emanation of previously cured malignancies of donors in SOTRs has been appertained to potential lack of tumor equilibrium in the transplant recipient due to immunosuppression that may have otherwise existed in the immunocompetent donor (Teng et al. 2008).

3.2 Role of Viral Infections in Carcinogenesis

Majority of cancers in SOTRs are driven by oncogenic viruses as SOTRs are more vulnerable to reactivation of latent infections as well as acquisition of new viral infections. Oncogenic viruses can trigger genomic instability, impair DNA (deoxyribonucleic acid) repair mechanism, disrupt cellular homeostasis, and alter cell signaling pathways abetting neoplastic transformation. The association between Human Papillomavirus (HPV) in anogenital cancers, Human Herpesvirus 8 (HHV8) in KS, and Epstein–Barr virus (EBV) in NHL and Hodgkin lymphomas (HL),

Merkel cell polyomavirus (MCPyV) in Merkel Cell Cancer of the skin, Hepatitis B virus (HBV) and Hepatitis C (HCV) viral infections in hepatocellular carcinoma has been well established. The International Agency for Research on Cancer (IARC) has in fact identified these viruses as biological human carcinogens (Bouvard et al. 2009). Innate and adaptive immune responses combat viral infections in an immunocompetent host and eliminate or minimize the severity of infections. Some infections may attain a latent state by restriction of gene expression and cessation of replication by subverting cell signaling pathways. Infections with oncogenic viruses in SOTRs could be transmitted from donor or may have new onset infection if not immune from prior exposure or vaccination and/or activated from dormancy after transplantation due to immunocompromised state. Oncogenicity of viral infections is mediated through direct or indirect carcinogenic mechanisms. Direct carcinogenic mechanisms include activation of proto-oncogenes, expression of viral oncogenes along with impairing tumor-suppressor genes leading to proliferation, angiogenesis, and resistance to apoptosis. Indirect mechanisms include promoting chronic inflammation and oxidative stress with production of mutagenic molecules leading to local inflammation and tissue damage, immunosuppression, chronic antigenic stimulation, and tumor growth modulation (Krump and You 2018; Saha et al. 2010).

3.3 Direct Effect of Immunosuppressive Agents in Carcinogenesis

Immunosuppressive drugs used in SOTRs are described to have carcinogenic potential independent of their effects on host immunity and exert direct carcinogenic effects. IARC has labeled immunosuppression drugs as human carcinogens and declared azathioprine and cyclosporine to be human carcinogens (IARC 1990, 2012). Cyclosporine promotes carcinogenesis independent of immunosuppression effects by various mechanisms including increased transcription and expression of the transforming growth factor-β (TGF-β) gene, which in turn promotes invasion and metastasis of tumor cells. In addition, cyclosporine also impairs response to DNA damage, inhibits apoptosis, and promotes vascularization of tumors by inducing vascular endothelial growth factor (VEGF) production (Barle et al. 2014; Hojo et al. 1999; Maluccio et al. 2003; Olshan et al. 1994; Yarosh et al. 2005). Azathioprine has direct carcinogenic effects and serves as a causative factor for development of premalignant dysplastic keratotic lesions. Among SOTRs on azathioprine regimen, higher levels of active metabolites of azathioprine were noted in red blood cells of transplant recipients with skin cancer compared to those without skin cancer. Metabolic derivatives of azathioprine can cause DNA damage and promote tumor growth in SOTRs and azathioprine is also reported to sensitize the skin to UV radiation (Lennard et al. 1985; Taylor and Shuster 1992).

4 Carcinogenesis in Immunocompromised Patients: Risk Factors

Israel Penn International Transplant Tumor Registry (IPITTR) is a SOTR tumor registry that is originally conceptualized by Dr. Israel Penn and was initially started at University of Colorado. This registry maintains a comprehensive repository of information on recipients of organ transplantation with cancers. This registry was previously known as Cincinnati Transplant Tumor Registry (CTTR) and was renamed to be IPITTR after Dr. Penn as a tribute to him. He was first to report high incidence of malignancies in SOTRs and his registry paved path for future research in this area (Israel Penn International Transplant Tumor Registry n.d.). Multiple studies based on this registry data and other population-based studies have reported several risk factors associated with the development of malignancy in SOTRs (Israel Penn International Transplant Tumor Registry n.d.; Acuna et al. 2016; Agraharkar et al. 2004; Chapman et al. 2013; Collett et al. 2010; Engels et al. 2011; Sherston et al. 2014; Wimmer et al. 2007). These risk factors can be primarily classified into patient related factors, transplant related factors, environmental factors, and management factors.

4.1 Patient Related Factors

Patient related factors including genetic predisposition, age, race, gender, comorbid medical conditions, underlying chronic pathology that necessitated the organ transplantation and prior history of infections with oncogenic viruses influence the risk of malignancy in SOTRs (Agraharkar et al. 2004; Buell et al. 2005; Chapman et al. 2013; Collett et al. 2010; Engels et al. 2011; Grulich et al. 2007; Krump and You 2018; Serraino et al. 2007; Vajdic et al. 2006; Wimmer et al. 2007). There is twofold increased risk of cancer for recipients >65 years of age, while children who receive organ transplantation carry a 15–30 times increased risk of cancer. There is an increase in the risk of cancer by 40% in SOTRs with prior history of cancer compared to those who do not have prior history of cancer. Caucasian race and male sex are associated with higher incidence of cancers. It was initially assumed that this increased risk may be related to higher rates of skin cancer due to inherent predisposition for skin cancers. Nonetheless, skin cancers alone could not validate the heightened risk that was observed. ESRD secondary to diabetic nephropathy pre-transplant are noted to have less cancer burden compared to other etiologies (Webster et al. 2007).

A retrospective study of large cohort of KTRs by Agraharkar et al., with a mean follow-up of 6.1 years with more than 10-year follow-up in 21% of the patients demonstrated high frequency of skin (40%), gastrointestinal (13%), urologic (11%) malignancies, and lymphomas (9%) compared to the general population. KTRs who developed malignancies seem to be older (43.5 years) at the time of transplant with a mean age of 50 ± 12 years at the time of cancer diagnosis (Agraharkar et al. 2004). In this study, incidence of breast and lung cancers was found to be lower in KTRs

than the general population with a SIR of 0.7. There is significant elevation in the incidence of lymphomas with a SIR of 4.9, renal cell cancers with a SIR of 7.2, and colorectal cancers with a SIR of 1.5. Although the risk of cancer development is higher in patients >60 years after transplantation with a relative risk (RR) of 6.2 compared to KTRs of age < 40 years, younger patients at the time of transplant were observed to have the highest relative risk for developing malignancies compared to age matched general population (Agraharkar et al. 2004). The similar risk profile for cancers had been reflected in patients with ESRD receiving dialysis therapy in a study by Maisonneuve et al. suggesting the importance of underlying disease contributing to the risk of cancers in transplant recipients (Agraharkar et al. 2004; Maisonneuve et al. 1999). EBV seronegative status at the time of transplant posed high risk for malignancy in SOTRs (Shahinian et al. 2003). There is notably high risk of gastric cancer related to Helicobacter pylori in Asian populations suggestive of genetic predisposition in the cancer manifestation (Engels et al. 2011).

4.2 Environmental Factors

Geographical and environmental factors play an important role in carcinogenesis. There is an exponential increase in risk of skin cancer in patients with high skin exposure compared to the regions with limited exposure to sun. This high risk is attributed to increased ultraviolet (UV) radiation associated with excess exposure to sunlight (Birkelans et al. 1995; Euvrard et al. 1997, 2003; Hartevelt et al. 1990; Kullavanijaya and Kim 2005; Vink et al. 1996). There are geographical differences in the spectrum of cancers in SOTRs as noted by a study that assesses cancer incidence in SOTRs in Taiwan. The skin cancer risk was noted to be significantly higher in western countries compared to the studies reported from Asian countries including Japan and Taiwan (Birkelans et al. 1995; Hartevelt et al. 1990; Hoshida and Aozasa 2004; Lee et al. 2016). Gastric and hepatocellular cancers are more common among SOTRs in Japan compared to western countries (Birkelans et al. 1995; Hartevelt et al. 1990; Hoshida and Aozasa 2004; Lee et al. 2016).

4.3 Transplant Related Factors

The role of transplant related factors such as type of organ transplant, time since transplantation, living versus deceased donor status and history of malignancy or oncogenic viral infections in carcinogenesis in SOTRs needs to be considered. There is an elevated risk of liver cancer among liver transplant recipients and this risk is more pronounced in the first 6 months after the transplant. There is substantially elevated risk of kidney cancers in KTRs with SIR of 6.6 [95% CI 6.12–7.32] and recipients of liver and heart transplant with SIR of 1.80 [95% CI 1.40–2.29] and 2.90 [95% CI 2.32–3.59], respectively (Engels et al. 2011). Cardiothoracic transplantation carries a higher burden of malignancy following transplant compared to other organs and may likely be related to higher intensity of immunosuppression used in

heart and lung transplant recipients (Collett et al. 2010; Engels et al. 2011; Na et al. 2013; Taylor et al. 2005a).

4.4 Management Related Factors

In SOTRs, time since transplantation, induction at the time of transplant, duration and intensity of immunosuppression are important factors that are well known to be associated with carcinogenesis. An increase in frequency of PTLD is noted in patients who received induction with antithymocyte globulin (ATG) or monoclonal anti-T cell antibody, muromonab-CD-3 (OKT3) (Cherikh et al. 2003). Agraharkar et al. reported a cumulative incidence of 19% for NMSC and 36% for all malignancies was reported in KTRs at 25 years after the transplant. Despite using a stringent p value of 0.002, post-transplant duration of >10 years had remained a significant risk factor in this study owing to the risk of prolonged exposure to immunosuppression therapy in development of cancers (Agraharkar et al. 2004). The high incidence of cancers in recipients who are of younger age at the time of transplantation compared to age matched controls could potentially be attributed to longer cumulative exposure to immunosuppressive therapy and likelihood of exposure to primary infections with oncogenic viruses after transplant compared to older counterparts who may have been exposed and achieved seronegative status prior to transplant (Saha et al. 2010).

5 Classification of Malignancies in SOTRs

Malignancy in SOTRs is a well-known complication and can be categorized into three broad groups: 1) Recurrence of cancers that were present before transplant and/or activation of dormant neoplasms, otherwise described as pre-transplant malignancy (PTM), 2) Cancers that are transmitted inadvertently from donors with prior history of malignancy or undiagnosed or occult malignancies at the time of transplant described as donor derived malignancy (DDM), and 3) Cancers arising de novo after the transplant reported as de novo malignancies (DNMs). In addition, latent infection with oncogenic viruses can predispose SOTRs to malignancy development after transplant in the setting of immunosuppression.

5.1 Recurrence of Pre-Transplant Malignancy in Solid Organ Transplant Recipients

Pre-transplant malignancy (PTM) is considered to be a significant risk factor for development of cancer in SOTRs. Analysis of IPITTR data suggested a recurrence rate of 21% with high frequency of recurrence in those who had been transplanted with a time interval <2 years since the diagnosis of cancer or receiving therapy for cancer (Penn 1997a). Contrary to data reported by Penn, a more recent meta-analysis

by Acuna et al. identified the risk of cancer recurrence to be lower in SOTRs with PTM than previously reported with a pooled recurrence rate of 1.6 [95% CI 1.0–2.6] per 100-person year. Recurrence rate of 1.1 per 100-person year was noted in liver transplant recipients compared to 2.4 in patients with KTRs (Acuna et al. 2017). A thorough evaluation including the risk of recurrent cancer is warranted in patients who are undergoing transplant assessment with prior history of malignancy. Cancer remission intervals and permissible wait times prior to considering for transplantation in these patients are variable based on the type of malignancy and survival expectancy from the neoplasm (Acuna et al. 2017). Multidisciplinary assessment with input from oncology colleagues is essential in the decision-making process. American Joint Committee on Cancer (AJCC) and American Society of Transplantation (AST) issued a consensus statement delineating the general recommendations to assist evaluation of patients undergoing evaluation for SOT with a history of PTM (Al-Adra et al. 2021). Nevertheless, the decision to either consider for transplant or defer the transplant may need to be tailored to each patient based on careful assessment of risk-benefit profile. Examination of various risk factors including tumor biology, response to treatment, cancer free interval, recurrent risk estimates, genetic and epigenetic risk factors, organ in consideration, potential effect of immunosuppression on recurrence of tumor, life expectancy and alterate therapy options is essential in analyzing the risk-benefit ratio (Al-Adra et al. 2021; Penn 1993).

5.2 Donor Derived Malignancy in Solid Organ Transplant Recipients

Transmission of cancers from donors with a history of previously treated cancer or undiagnosed cancer is an infrequent cause of cancer in SOTRs (Feng et al. 2002; Kauffman et al. 2000; Ma et al. 2014; Penn 1995). The estimates of risk are variable with significantly higher rates in IPTRR registry compared to Organ Procurement and Transplantation (OPTN) reports. Magnitude of risk varies based on the type of cancer and transplanted organ. Donors with a history of primary central nervous system tumors, renal cell carcinoma, malignant melanoma, and choriocarcinoma are at high risk for transmission compared to colon and breast cancers (Penn 1995). The persistent disparity between organ donation and end stage organ failure patients awaiting organs leads to evolution of extended criteria for organ donation. The expansion of donor pool by including older age donors renders high risk for donor derived malignancy (DDM) as advanced age is associated with a high rate of premalignant or occult lesions. Primary central nervous system malignancies are reported to be a common source of DDM. Careful assessment of donors with unusual presentations and prior history of malignancies is essential to minimize the risk of transmission. United States Donor Transmitted Assessment Committee (DTAC) provides guidance to physicians and patients regarding risk of donor transmitted diseases including malignancy from potential donors. Risk categorization of transmission risk of malignancies is a helpful aid in assessing the potential risk of transmission (Ison and Nalesnik 2011; Kauffman et al. 2002; Penn 1995).

Intra-operative assessment of all visible organs and prompt biopsy and histological assessment of any suspected masses and/or lymph nodes must be considered. Once a DDM is identified in an organ recipient, notification of other organ recipients from the donor is crucial.

5.3 De Novo Malignancies in Solid Organ Transplant Recipients

De novo malignancy is a well-recognized complication following organ transplantation due to inherent need for immunosuppression to prevent graft rejection and has emerged as a major cause of mortality and morbidity in these patients. De novo malignancies in SOTRs are primarily driven by immunocompromised state due to pharmacologic immunosuppression, oncogenic viral infections, direct oncogenic effects of immunosuppression, genetic and environmental risk factors. A 10-year incidence of de novo cancers in SOTRs is twice that of an age- and sex- matched general population cohort with marked elevation in the incidence of NMSC (Collett et al. 2010). A retrospective analysis of data from cardiothoracic and liver transplant recipients between 1984 and 2006 in Australia demonstrated excess risk of death secondary to de novo malignancy compared to the general population (Na et al. 2013). This risk was consistently elevated in both sexes, pediatric and adult populations and in all transplanted organ groups. Most common malignancy that resulted in death of transplant recipient was NHL in this cohort. Pediatric transplant recipients were noted to have the highest risk of death from de novo malignancy with 80% of deaths in pediatric SOTRs were related to NHL (Na et al. 2013). Age is an important risk factor in estimating the excess cancer risk. Like in the general population, advanced age is a risk factor for cancer development in SOTRs. While the absolute risk of cancer is significantly elevated in older transplant recipients, excess rate of cancer risk compared to the general population, defined by the relative risk (RR) is much greater in youngest recipients of organ transplant (Chapman et al. 2013; Engels et al. 2011; Na et al. 2013; Webster et al. 2007). This may be secondary to increased vulnerability of young transplant recipients to primary infections in the setting of chronic employment of immunosuppression. Heart and lung transplant recipients are at the higher risk for de novo malignancy after transplantation compared to kidney and liver transplant recipients owing to the risk of more intense immunosuppression required in cardiothoracic transplantation (Na et al. 2013).

Most common post-transplant malignancies in SOTRs include NMSC, lymphoproliferative disorders, KS, and HPV related anogenital cancers (Acuna et al. 2016; Chapman et al. 2013; Collett et al. 2010; Engels et al. 2011; Na et al. 2013; Webster et al. 2007). Although overall cancer burden is increased in SOTRs, the incidence of breast, prostate, ovarian, and testicular cancers in SOTRs is not elevated in comparison with the general population (Wong et al. 2017). Population-based studies in patients with HIV/AIDS noted that the incidence of breast, prostate, and ovarian cancers is observed at a relatively low or comparable rate to that of the general population, suggesting that an immunocompromised state in itself may not predispose to the increased number of these cancers. Nonetheless, breast cancers are

associated with poor prognosis in SOTRs compared to the general population, despite similar incidence rates (Grulich et al. 2007; Na et al. 2013; Serraino et al. 2007).

The risk of death from de novo malignancies is elevated in all SOTRs compared to the general population. The magnitude of cancer risk differed among SOTRs based on the transplanted organ with excess risk greatest for lung transplant recipients (Acuna et al. 2016; Chapman et al. 2013; Engels et al. 2011; Na et al. 2013). Compared to the general population, prognosis of cancers in SOTRs is poor and is associated with excess mortality. Cancers are more advanced at the time of diagnosis in SOTRs with more poorly differentiated tumors and respond poorly to the treatment (Acuna et al. 2017; Ajithkumar et al. 2007). Interactions between immunosuppressants and antineoplastic agents need to be considered in designing treatment strategies. A multidisciplinary approach may need to be pursued to address these cancers in SOTRs.

5.3.1 Skin Cancers

The most common cancer in SOTRs is NMSC with predominance of squamous cell carcinoma (SCC) of the skin with >50-fold increased risk compared to that of the general population (Euvrard et al. 1997, 2003; Hartevelt et al. 1990; Krynitz et al. 2013; Na et al. 2013; Penn 1997b). Although keratinocyte carcinomas including SCC and basal cell carcinoma (BCC) account to >90% of skin cancers in SOTRs, KS, Merkel cell carcinoma, and malignant melanoma are reported to occur more commonly in SOTRs compared to the general population. There is reported 65–250-fold increase in the incidence of SCC and 10–16-fold increased incidence of BCC in SOTRs. In contrast to the general population, the ratio of SCC to BCC is reversed in SOTRs (Euvrard et al. 2003; Penn 1997b). SCCs of SOTRs are noted to have histologic features suggestive of epithelial to mesenchymal transition that is ascribed to the use of immunosuppression (Euvrard et al. 2003).

Direct effects of immunosuppression agents, type and duration of immunosuppression, and exposure to UV radiation play a central role in development of skin cancers (Han et al. 2012; Krynitz et al. 2013; Penn 1997b; Vink et al. 1996; Yarosh et al. 2005). Caucasian race, older age at the time of transplant, exposure to HPV infections, and history of prior skin cancers contribute to the risk of skin cancer in SOTRs. Genetic factors such as human leukocyte antigen (HLA) and polymorphisms in glutathione *S*-transferase may also influence development of cutaneous neoplastic lesions. SOTRs with cutaneous carcinomas have significantly lower CD4 counts than patients without skin cancer (Banvinck et al. 1993; Euvrard et al. 2003; Harwood et al. 2000; Krynitz et al. 2013; Ramsay et al. 2001). Higher incidence of skin cancers is noted in heart transplant recipients compared to KTRs and liver transplant recipients. However, this differential risk is attributed to higher intensity of immunosuppression used in heart transplant recipients. Skin cancers are more prevalent in geographical areas with high sun exposure and cancers are seen more often in sun exposed body parts in SOTRs. UV light has direct carcinogenic effect and causes local immunosuppression by mutagenic effects on *p*53 tumor-suppressor gene (Banvinck et al. 1993; Euvrard et al. 2003; Vink et al.

1996; Yarosh et al. 2005). UV light also induces histologic changes locally, promotes local inflammation, and has synergistic effect with HPV and immunosuppression agents (Harwood et al. 2000; Krynitz et al. 2013). There is also a significant association between SCC and HPV infection. HPV is postulated to be cocarcinogenic and HPV DNA has been isolated in approximately 65-90% of SCC lesions in SOTRs (Euvrard et al. 2003; Harwood et al. 2000; Krynitz et al. 2013). SCC in SOTRs appears to be more aggressive with high metastatic potential compared to the general population. Presence of multiple tumors, extracutaneous manifestation of tumors, cephalic location, older age, and high exposure to UV radiation are associated with unfavorable prognosis (Euvrard et al. 2003; Krynitz et al. 2013).

5.3.2 Lip Cancer

SOTRs are at greater risk for lip cancer with 13–66-fold increase in risk compared to the general population with poorly understood reasons for this excess risk (Acuna et al. 2016; Chapman et al. 2013; Collett et al. 2010; Engels et al. 2011; Krynitz et al. 2013). A 15-fold increase in the incidence of lip cancer is observed in SOTRs compared to the general population (Laprise et al. 2019). While elevated risk of lip cancer is noted both in SOTRs and patients with HIV/AIDS, the magnitude of risk is higher in SOTRs compared to patients with HIV/AIDS (Grulich et al. 2007; Laprise et al. 2019). Lip cancers are predominantly SCCs and can be external lip cancers or mucosal lip cancers. Tobacco use and alcohol consumption predisposes to mucosal lip cancers and exposure to UV radiation plays an important role in development of cancers of the external lip (Euvrard et al. 2003; Grulich et al. 2007; Laprise et al. 2019). Prior diagnosis of SCC, Caucasian race, immunosuppressive therapy particularly with cyclosporine and/or azathioprine have been strongly associated with lip cancer (Laprise et al. 2019).

5.3.3 Kaposi Sarcoma

KS is an angioproliferative disorder of vascular endothelium driven by oncogenic virus HHV-8. Although most cases of KS in SOTRs are secondary to HHV-8 reactivation in organ recipients, cases of donor transmission have been described in the literature. The incidence of KS in SOTRs is profoundly increased and is 400–500-fold greater in SOTRs compared to the general population with a preponderance for male sex (Acuna et al. 2016; Chapman et al. 2013; Collett et al. 2010; Engels et al. 2011; Krynitz et al. 2013). It can manifest as a cutaneous sarcoma or visceral sarcoma or can present with mixed features in SOTRs. The most common presentation of cutaneous KS is angiomatous lesions on legs similar to classic KS and gastrointestinal tract, lymph nodes, and lung are affected in visceral form of KS. The incidence of KS is greatly elevated in SOTRs compared to the general population and predominantly seen in patients of Mediterranean, Jewish, Arabic, Caribbean, and African descent. KS manifests at an earlier age in SOTRs compared to patients with classic KS with a mean age of 43 at the time of diagnosis in SOTRs (Euvrard et al. 2003). KS is known to respond well to reduction in immunosuppression and especially reduction of CNIs to the minimal safe dose is associated with regression of lesions.

5.3.4 Anogenital Cancers

Immunocompromised patients are at increased risk for anogenital cancers, and the risk increases by approximately 20-fold in these patients compared to the general population. HPV infection, multiple sexual partners, smoking, prior history of genital herpes, presence of extragenital skin cancers, and high intensity immunosuppression are all risk factors associated with development of anogenital cancer (Euvrard et al. 2003). Anogenital HPV is highly prevalent in female transplant recipients who are sexually active (Euvrard et al. 2003).

5.3.5 Post-Transplant Lymphoproliferative Disorders

Post-transplant lymphoproliferative diseases (PTLD) is used to describe a spectrum of lymphoproliferative disorders ranging from benign hyperplasia to aggressive lymphomas in SOTRs. PTLD is the most common cause of cancer related death in both adult and pediatric organ transplant recipients (Campistol et al. 2012; Vegso et al. 2007). Although proliferation of any cell lines B cells, T cells, natural killer cells, and plasma cells could cause PTLD, vast majority of PTLDs are of B cell lymphomas and a strong association with EBV infection has been noted. PTLDs in SOTRs are more aggressive in nature and respond poorly to conventional treatment measures compared to lymphoproliferative malignancies in the general population. Vast majority of PTLDs (90%) are associated with EBV infection (Opelz and Dohler 2004; Shahinian et al. 2003; Taylor et al. 2005a; Yarosh et al. 2005). Normally functioning T cell plays a critical role in immune control of EBV infection and inhibition of T cell function secondary to immunosuppression in SOTRs and impaired T cell function in primary immunodeficiency disorders and patients with HIV/AIDs leads to loss of immune control of EBV infection. Risk of NHL is elevated in these conditions due to loss of immune modulation of EBV mediated lymphoproliferation (Opelz and Dohler 2004).

NHL usually demonstrates bimodal incidence pattern with early onset PTLD developing within the first year of the transplant and late onset PTLD developing later in the post-transplant course with a median time of 4 years (Opelz and Dohler 2004; Shahinian et al. 2003). Recipients of heart and lung transplants are at a higher risk of PTLD than KTRs and liver transplant recipients owing to the need of heavy immunosuppression in the former group. SOTRs who are induced with T cell depleting agents such as antithymocyte globulin (ATG) or muromonab-CD-3 (OKT3) were observed to have higher risk of developing PTLD (Gao et al. 2003; Opelz and Dohler 2004). Heightened incidence of PTLD is noted in cardiothoracic transplant recipients compared to KTRs and liver transplant recipients (Cherikh et al. 2003; Gao et al. 2003). Tacrolimus is associated with higher risk of PTLD than cyclosporine and patients treated with mycophenolate mofetil (MMF) are reported to have less risk of PTLD compared to patients treated with azathioprine.

The risk of Hodgkin's lymphoma (HL) is also elevated in SOTRs as well as those with HIV infection when compared to the general population, indicating the role of impaired immune regulation in the inception of this cancer. Analysis of SRTR data by Quinlan et al. demonstrated a twofold increase in risk of developing HL in SOTRs compared to general population with a SIR of 2.2 [95% CI 1.7–2.7] with

greatest risk in children and adolescents. EBV seronegative status at the time of the transplant was associated with elevated risk with SIR of 4.7 [95% CI 1.9–9.6]. Cardiothoracic transplant recipients were noted to be at higher risk for HL development than abdominal organ recipients (Quinlan et al. 2010).

5.3.6 Thyroid Cancer

SOTRs are at elevated risk for thyroid cancer and a 2.5-fold higher incidence rate is noted in SOTRs compared to the general population. Risk was amplified in KTRs with an incidence rate ratio (IRR) of 1.26 [95% CI 1.03–1.53]. The risk is more pronounced in patients who underwent kidney transplant secondary to hypertensive nephrosclerosis with an IRR of 1.41 [95% CI 1.03–1.94] and liver transplant secondary to cholestatic liver disease/cirrhosis with an IRR of 1.69 [95% CI 1.09–2.63]. In addition, longer duration of dialysis prior to kidney transplant is strongly associated with higher incidence of thyroid cancer. Majority of thyroid cancers in SOTRs were identified to be papillary thyroid cancers (91%) followed by follicular cancers (5%). Increased risk of death with a Hazard Ratio (HR) of 1.33 [95% CI 1.02–1.73] is noted among patients diagnosed with thyroid cancer following the organ transplantation (Kitahara et al. 2017).

5.3.7 Lung Cancer

Lung transplant recipients are at highest risk for lung cancers among SOTRs. Despite lower incidence of lung cancer in kidney, liver, and heart transplant recipients compared to lung transplant recipients, the overall risk of all SOTRs is higher than general population. The risk of developing lung cancer is sixfold higher in lung transplant recipients compared to two- to threefold increased risk noted in the recipients of other organs. Smoking is a major risk-factor in the development of lung cancer. The risk of lung cancer is higher in single lung transplant recipients compared to bilateral lung transplant recipients (Collett et al. 2010; Engels et al. 2011). This may be attributed to the presence of native lung in single lung transplant recipient that continues to carry the burden of underlying disease process, and the exposure to the risk factors that may have been contributed to the pathogenesis of underlying disease process that prompted the transplant.

6 Immunosuppression in Organ Transplantation

Immunosuppressive medications used in organ transplantation are associated with a wide spectrum of adverse effects including malignancy and can contribute to decreased life expectancy or quality of life in these patients. First successful life prolonging kidney transplantation was performed in 1954 between identical twins at Peter Bent Brigham Hospital, Massachusetts. The genetic matching of recipient and donor ushered the graft and recipient survival despite no use of immunosuppression. Sublethal total body irradiation (TBI) by Murray et al. demonstrated that immunologic barrier of transplantation could be vanquished by immunosuppression. Nonetheless, cytoablative radiation has proven to be an undesirable modality of

immunosuppression due to high mortality (>90%) associated with TBI. Scientific work of Sir Peter Medawar laid the foundations of transplant immunology with discovery of acquired immunological tolerance and received Nobel Prize for his pioneering work. Pharmacologic immunosuppression gained momentum in the 1960s with emergence of therapeutic agents for leukemia such as 6-mercaptoprurine, cyclophosphamide, and methotrexate. George Hutching and Gertrude Elion introduced azathioprine, a more clinically permissible congener of 6-mercaptopurine. Sir Roy Clane's work resulted in the emergence of azathioprine as a successful immunosuppression therapy and a viable therapeutic option for organ transplant recipients. A significant survival advantage was noted with combination immunosuppressive regimens comprising of azathioprine and corticosteroids. Continued evolution of science in the field of transplant led to the discovery of cyclosporine compared to single agent regimens. Dramatic graft and patient survivals were noted following the use of cyclosporine and brought transformational change in field of organ transplantation.

Immunosuppressive regimens are essential in preventing rejection and for the survival of allograft in SOTRs. Most organ transplant recipients receive a combination of two or three pharmacologic agents for immunosuppression. Although some immunosuppressant medications are described to be more carcinogenic than others, it is the overall intensity and duration of immunosuppression that profess the risk of cancer development in SOTRs (Cherikh et al. 2003; Herman et al. 2001; Martinez and de Gruijl 2008; Taylor et al. 2005b). Corticosteroids are used as first-line agents during the transplantation and immediately after transplantation. Corticosteroids are anti-inflammatory and decrease the production of cytokines and circulating CD4 cells. Corticosteroids promote carcinogenesis predominantly through immune modulation. Steroids reduce the immune surveillance of tumor cells resulting in evasion and escape of tumorigenic cells (Taylor et al. 2005b). Corticosteroids also increase the risk of infection and thus cancers related to oncogenic viruses.

MMF and azathioprine are antimetabolites that are used in organ transplantation. Azathioprine has been recognized as a carcinogen and is implicated in the development of skin cancers and NHL in SOTRs. The use of azathioprine in modern era of organ transplantation is sparse. Synergistic effects of azathioprine and UV radiation result in mutagenic oxidative damage of DNA and impaired repaired response leading to carcinogenesis. Despite the pro-oncogenicity seen in in vitro studies with impaired DNA damage response and inflated invasion of tumor cells, clinical studies failed to demonstrate any substantial increased risk of malignancy with MMF. In fact, MMF based immunosuppressive regimens demonstrated lower risk of PTLD compared to immunosuppressive regimens based on azathioprine (Cherikh et al. 2003).

Cyclosporine and Tacrolimus are the two common CNIs used in the management of SOTRs and CNIs remain cornerstone of immunosuppression in SOTRs. CNIs based maintenance immunosuppressive regimens are associated with reduced graft rejection and improved survival. However, unfavorable nephrotoxic and metabolic side effect profile of CNIs led to investigations toward CNI free immunosuppressive regimens. CNIs based immunosuppressive regimens are also implicated in increased

malignancy risk in SOTRs. CNIs can promote carcinogenesis through immunosuppression as well as direct carcinogenic effects by inducing TGF-β production that aids in evasion of host immune defenses and stimulating the secretion of vascular endothelial cell growth factor (VEGF) that facilitates tumor angiogenesis (Han et al. 2012; Hojo et al. 1999; Olshan et al. 1994). Tacrolimus has a dose-dependent effect on TGF-β expression and thus permits the idea of potential modulation of carcinogenic effect with therapeutic level monitoring.

Mammalian Target of Rapamycin (mTOR) is a conserved protein kinase that plays an important role in cell growth, proliferation, survival, metabolism, and autophagy through various signaling pathways. The mTOR signaling pathway modulates protein synthesis, gene transcription, and translation and thus controls cellular homeostasis, angiogenesis, cytoskeletal remodeling, stress response, and activity of immune cells. It plays a key role in activation, differentiation, and function of immune cells by regulating the expression of various inflammatory mediators, cytokines, chemokines, membrane receptors, and apoptosis (Koehl et al. 2004; Martinez and de Gruijl 2008). Dysregulation of various elements of this pathway could lead to disease states such as neoplastic transformation, insulin resistance, obesity, and neurodegeneration. mTOR inhibitors piqued interest in transplant field as these agents offer immunosuppression and tumor growth suppression. There are several clinical trials that demonstrated reduced incidence of cancers in patients treated with sirolimus in kidney transplant recipients (Alberu et al. 2011; Gatault and Lebranchu 2013; Lebranchu et al. 2009; Schena et al. 2009). CONCEPT study demonstrated less incidence of cancers in patients whose immunosuppression was switched from Cyclosporine to Sirolimus 3 months after kidney transplantation compared to the cohort that continued to receive CNI-based immunosuppressive therapy (Lebranchu et al. 2009).

7 Conclusions

Optimal immunosuppression is key to the success of organ transplant. However, chronic exposure to immunosuppression in SOTRs is unfortunately associated with higher incidence of various hematologic and non-hematologic malignancies. Complex interplay of various factors including immune, non-immune, infectious, environmental, and genetic factors leads to carcinogenesis in SOTRs. While advances in transplant medicine, histopathology, and surgery have helped in expanding the donor pool and willingness to take more risk, they come with the cost of increased risk of cancers, thus suggesting the need for enhanced vigilance in screening, patient and donor selection, early recognition and management of malignancies, as well as individualization of appropriate immunosuppressive regimens in this high-risk population.

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Adverse Effects of Immunosuppression: Nephrotoxicity, Hypertension, and Metabolic Disease

Jamael Hoosain and Eman Hamad

Contents

Abstract

The use of Immunosuppression has led to the tremendous improvement in graft survival. However, immunosuppressants have been found to cause a variety of metabolic derangements including but not limited to: insulin resistance and diabetes, hyperlipidemia, hypertension, and weight gain after transplantation. This combination of metabolic risk factors may be associated with increased cardiovascular disease (Grundy et al., Circulation 112(17):2735, 2005). In addition many transplant recipients may have many of these risk factors pre-transplant

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that are exacerbated by immunosuppression. These facts emphasize the need for rigorous follow-up and management of these risk factors post-transplant.

The most common immune suppressant regimens may include different combinations of these agents: Corticosteroids, Calcineurin inhibitors (CNIs), Mammalian Target of Rapamycin (mTOR) Inhibitors, Antimetabolite.

Keywords

Immunosupression · Metabolic disease · Nephrotoxicity

1 Introduction

The cornerstone of heart transplant recipient care comes in the form of meticulous management of immune modulation to ensure immunologically acceptance of the donor organ. Recipient survival requires an equilibrium between acute rejection and the adverse effects from chronic immunosuppression. In this chapter, we will discuss immunosuppression's effects on nephrotoxicity, hypertension, and metabolic consequences that transplant recipients might face.

2 Immunosuppression and Nephrotoxicity

There are many challenges in managing immune modulation. A significant long-term consequence of immunosuppression is nephrotoxicity. The nephrotoxicity and significant progression of renal dysfunction resulting from these agents has proven to be an Achilles' heel in heart transplantation. Based on the ISHLT registry data, between the years January 1995 to June 2017, 6.7% of transplant survivors experienced severe renal dysfunction (defined as serum creatinine >2.5 mg/DL, chronic dialysis, or renal transplantation) within the first year, as well as observed in 15.7% of survivors within 5 years, and 22.3% within 10 years (Khush et al. 2019). In the current era, freedom from severe renal dysfunction has improved compared to the previous era (1995–2004), however severe renal dysfunction still provides a significant impact on survival to 1 year. There are many factors that contribute to development of severe renal dysfunction after transplant. The recipient's pre-transplant creatinine plays a role in which there is an inflection point in which the hazard ratio increases for serum creatinine >1.1 mg/dL. In this section we will be exploring in more detail each of the immunosuppressant classes and their effects on renal function (Khush et al. 2019).

2.1 Corticosteroids

Corticosteroids or glucocorticoids are some of the most widely used immunosuppressant agents. They are non-specific anti-inflammatory agents that work at the

level of the nucleus to augment expression of pro-inflammatory proteins. Corticosteroids are associated with many adverse effects with long-term use, however there is no reported direct nephrotoxicity associated with their use.

2.2 Calcineurin Inhibitors

Calcineurin inhibitors have remained at the cornerstone of maintenance immunosuppression since the early 1980s. The current available calcineurin inhibitors (CNIs) are cyclosporine (CsA; Sandimmune, Gengraf, or Neoral) and tacrolimus (Tac; Prograf). Both cyclosporine and tacrolimus block calcium activated calcineurin. Specifically, cyclosporine binds to cyclophilin and tacrolimus to the FK binding protein-12. Each drug and their respective immunophilin binds to calcineurin. Calcineurin is a phosphatase that dephosphorylates multiple molecules including nuclear factor of activated T cells (NFAT), which will subsequently bind premotor regions on several cytokine genes causing up-regulation. Cyclosporine and tacrolimus both inhibit calcineurin, and thus blunt the upregulation of the cytokines including interleukin-2 (IL-2). Additionally, cyclosporine also stimulates transforming growth factor beta (TGFB) production which also contributes to its immunosuppressive effects (Shin et al. 1998).

Cyclosporine has been part of the cornerstone of immunosuppression since the early 1980s. Over the years, different formulary have existed including a modified microemulsion formulation of cyclosporine which demonstrated a greater bioavailability and more predictable pharmacogenetics and oil-based preparations (Cooney et al. 1998). Nephrotoxicity is a well-known associated consequence of cyclosporine use. Nephrotoxicity with cyclosporine is a dose-related effect that can either be acute or chronic resulting in arteriolar sclerosis and tubular interstitial fibrosis. In rare instances cyclosporine may also manifest as a hemolytic uremic syndrome (Valantine 2000).

Tacrolimus, formally known as FK506, is the other calcineurin inhibitor that is widely used. There have been a number of studies comparing tacrolimus to cyclosporine, in both the oil-based and micro emulsion formularies. Tacrolimus was compared to oil based cyclosporine, there were similar patient and allograft survival, however the incidence of moderate to severe cellular rejection at 6 months was significant lower in the tacrolimus group compared to the micro-emulsion cyclosporine group (Taylor et al. 1999; Reichart et al. 2001; Grimm et al. 2006; Kobashigawa et al. 2006).

In a cohort study of solid organ transplants, including heart, liver, and lung in the USA, the overall incidence of CKD was 60% in the cyclosporine group and 20% in the tacrolimus group. At a median follow-up of 36 months, 17% had developed CKD stage IV or greater. The risk of CKD increases over time with all of the solid organ transplant and was associated with a 4.6 fold increase in risk of death compared to those without CKD. Twenty-nine percent of patients went on to develop end-stage renal disease requiring renal replacement therapy. Risk factors had included older age, lower pretransplant GFR, female sex, postop AKI, baseline

history of diabetes and hypertension as well as hepatitis C viral infection (UTD 12, 14). Other risk factors include concomitant nephrotoxic drug use such as nonsteroidal anti-inflammatory drugs, as well as drugs that inhibits the cytochrome P450 3A4/5 thereby increase exposure to CNI metabolites and drugs that inhibited P glycoprotein mediated efflux of TNI from tubular epithelial cells causing an increase in local renal exposure (UTD 31). Polymorphisms in the genes encoding for CYP 3 A4/5 and P glycoprotein (ABCB1) will also affect the risk of nephrotoxicity (UTD 31).

Both acute and chronic nephrotoxic effects are generally similar with cyclosporine and tacrolimus (UTD 15). A lot of data in this field comes from renal transplant recipient and involves cyclosporine given the wide availability. Acute calcineurin inhibitor nephrotoxicity is due to vasoconstriction of the afferent and arterial, thereby causing a reduction in renal blood flow and glomerular filtration rate (UTD 36). Although the exact mechanism is still unclear, there seems to be a substantial impairment of endothelial cell function with enhanced release of vasoconstrictors like endothelin and thromboxane (UTD 36). Renal plasma flow and GFR reduction correlate with the dose and peak cyclosporine level, which may be associated with increased urinary excretion of endothelin, which decreases when trough drug levels are reached (UTD 38). This increase in vascular resistance may be reflected by an increase in plasma creatinine concentration as well as systemic hypertension (UTD 2). This vasoconstriction prevents and delays recovery from early AKI associated with hypoperfusion and ischemia. It is also possible that these episodes of renal ischemia may contribute to a chronic cyclosporine nephrotoxicity.

In addition to the aforementioned effects on renal function, there are a number of electrolyte and metabolic abnormalities that may result from CNI use. These include hyperkalemia, non-anion gap metabolic acidosis, hypomagnesemia, hyperkalemia, hypophosphatemia, hyperuricemia and gout (UTD 20, 21, 67, 68).

CNI use may cause an elevation in plasma potassium concentration by reducing the efficiency of urinary potassium excretion by decreasing the activity of the renin-angiotensin-aldosterone system and by impairing tubular responsiveness to aldosterone (UTD 69, 70). There are some in vitro studies suggesting that cyclosporine may directly impair the function of the cells in the cortical collecting tubule, with decreased activity of the NA–K–ATPase pump with inhibition of the luminal potassium channel and thereby increasing chloride reabsorption (UTD 70). Tacrolimus has a similar inhibitory effect on the Na–K–ATPase pump (UTD 73).

Cyclosporine can cause tubular injury thereby impairing acid excretion as well as decreasing aldosterone activity with suppression of ammonia excretion by the concomitant hyperkalemia (UTD 2, 70). Cyclosporine may also cause urinary phosphate wasting, as well as abnormalities in calcium and magnesium reabsorption resulting in hypophosphatemia, hypomagnesemia, and hypocalcemia respectively (UTD 2, 78). The CNI effects on glomerular and tubular function can also lead to a decrease in uric acid excretion leading to hyperuricemia (UTD 67).

As mentioned, CNI nephrotoxicity can manifest either as an acute or chronic injury. Other renal effects include tubular dysfunction and rarely a thrombotic microangiopathy which can also lead to acute renal loss. The majority of data

published on this topic comes from cyclosporine, however there are similar patterns of renal disease described and tacrolimus, thus implying a drug class effect. (UTD 2–5).

One of the best ways to prevent chronic calcineurin inhibitor induced nephrotoxicity is to minimize the patient's exposure to CNI agents, and potentially replace them with non-nephrotoxic immunosuppressive agents. Strategies for minimizing CNI exposure are in the section below. There has been a great deal of interest in finding other agents to minimize the nephrotoxic effects of CNI. These include cold fish oil (UTD 85, 86, 90), renin angiotensin system inhibitors, calcium channel blockade, thromboxane synthesis inhibitors (UTD 111), and pentoxifylline (UTD 110), however agents have either unproven or no benefit.

There are some small animal studies that showed ACE inhibitors and angiotensin receptor blockers can prevent cyclosporine-induced interstitial fibrosis and improve renal function, however studies in humans have not demonstrated a clear benefit (UTD 100–102) Similar findings and interval studies are suggestive that aldosterone antagonism with spironolactone may be beneficial, however there are no human studies to support this. (UTD 105).

Unlike RAS inhibition, calcium channel blockers in animal and human data in renal transplants suggest that concomitant administration with cyclosporine may be protective by minimizing the renal vasoconstriction (UTD 92). Although there may be some small benefit in renal vasoconstriction initially in renal transplant recipients, there is no proven benefit in long-term outcomes of graft survival, or chronic vascular and tubulointerstitial injury. There has also been an inability to demonstrate a better outcome in protecting against kidney injury with calcium channel blockers versus ACE inhibitors (UT 50).

2.3 Antimetabolites

The antiproliferative agents otherwise known as antimetabolite provide an additional component of the backbone of maintenance immunosuppression. Their fax can be found by interference of the synthesis of nucleic acids, preventing the proliferation of T and B lymphocytes. The two most commonly encountered antimetabolites in heart transplantation include azathioprine and mycophenolic acid.

2.4 Azathioprine

Azathioprine (Imuran), once hydrolyzed and converted to its purine analog thioinosine-monophosphate, becomes incorporated into DNA inhibiting the mitotic and proliferative function of activated T and B lymphocytes. This drug used to be a common addition to a calcineurin-based regimen for maintenance immunosuppression. Side effects include leukopenia, hepatotoxicity, and pancreatitis amongst others, however nephrotoxicity is not one of them.

2.5 Mycophenolic Acid

In this current era, mycophenolic acid has replaced azathioprine as the preferred antimetabolite agent. The two major forms are mycophenolate popliteal (MMF; CellCept) or mycophenolate sodium (Myfortic). Mycophenolate mofetil also is a prodrug requiring hydrolysis to its active form of mycophenolic acid (MPA), while mycophenolate sodium (EC–MPS) is an enteric-coated, delayed release salt of mycophenolic acid. In its mycophenolic acid form it becomes a reversible inhibitor inosine monophosphate dehydrogenase, preventing de novo synthesis of guanine nucleotide, and thus selectively inhibiting T and B lymphocyte proliferation. There are a number of benefits to mycophenolic acid over azathioprine including less bone marrow suppression, amongst others that will be discussed in other parts of this book. Both forms of mycophenolic acid discussed are not nephrotoxic.

2.6 Proliferation Signal Inhibitors/Mammalian Target of Rapamycin (mTOR) Inhibitors

The fourth class of immunosuppressive agents include the proliferation signal inhibitors (PSI) otherwise known as the mammalian target of rapamycin (mTOR) inhibitors. There are a few indications that have risen to the top in which PSI based regimens have proven ineffective over the more conventional maintenance therapies.

The two drugs in this category of medications include sirolimus (Rapamune) and everolimus (Zortress). Similar to tacrolimus they bind to the FK binding protein, but rather than blocking calcineurin dependent T-cell activation, there is inhibition of a protein kinase in the cytoplasm called the mammalian target of rapamycin (mTOR). TOR phosphorylases proteins that regulate the cell cycle, thus it plays a critical role in transmission of the IL-2 mediated growth and proliferation of T and B lymphocytes. The activation of TOR also plays a role in the proliferation of smooth muscle and endothelial cells and may explain some of the benefits and prevention of graft atherosclerosis and indication of tumor growth in animal models (Heitman et al. 1991; Guba et al. 2002; Poston et al. 1999). Inhibition of mTOR will inhibit T and B-cell proliferation in response to cytokine signaling.

Sirolimus or Rapamune has been compared to a number of standard immunosuppressive agents, notably, as an alternative to azathioprine in a prospective, open label, randomized trial in which there was a lower proportion of moderate to severe acute cellular mediated rejection episodes within 6 months and the sirolimus group out as well has a reduction in cardiac allograft vasculopathy at both 6 months and 2 years (Kirklin et al. 1994). This made sirolimus an attractive agent. Everolimus (Zortress) is an analog of sirolimus, with the main difference being everolimus's better bioavailability with a shorter terminal half-life (30 h), compared to sirolimus (60 h) (Klawitter et al. 2015).

Compared to CNIs, PSIs are unique and that they inherently have no nephrotoxicity effect but can potentiate the efficacy and nephrotoxicity of CNIs. When a CNI + PSI-based strategy is used, a dose reduction in the CNI of at least 25% is

recommended. Sirolimus and everolimus have similar toxicity profiles overall, and though no head-to-head comparison had been made between the two drugs (Keogh et al. 2004).

2.7 Induction Therapy

Induction therapy is a widely available treatment strategy in which early intense immunosuppressant agents are administered in the early post-op period, with a goal of providing immediate immunomodulation. Approximately only 40–50% of heart transplant programs currently employed this strategy of upfront intense immunomodulation when risk of allograft rejection is the highest (Khush et al. 2019). There are a few different immunogenic targets which can be utilized, such as monoclonal antibody against IL-2 receptor antagonism (currently available agent, basiliximab (Simulect)), polyclonal anti-thymocyte antibodies (ATGAM-horse derived lymphocyte immune globulin or Thymoglobulin-rabbit derived lymphocyte immune globulin) and antibodies against the CD52 antigen (currently available agent, Alemtuzumab (Campath-1H)). Induction strategies may benefit those who are at high risk for severe rejection, such as African-American patients, younger patients, those with high levels of preformed antibodies, and in cross matches with a high number of HLA mismatches (Rosenberg et al. 2005). Induction allows for a secondary advantage of delaying initiation or lower doses of other immunosuppressant agents, namely those with significant adverse effects such as those that induce nephrotoxicity or metabolic sequelae (Cantarovich et al. 2004; Higgins et al. 2005). In a small study from Cantarovich et al., they studied postop renal dysfunction in heart transplant recipients receiving ATG induction, which the authors results suggested that delaying cyclosporine initiation post induction had comparable survival at 1 year, with a reduction in post-op renal dysfunction (Higgins et al. 2005). In a Cochrane review from Penninga et.al, reviewing 22 randomized control trials that utilize T-Cell antibody, the authors concluded that there was no clear benefit or harm associated with use of any kind of T-cell antibody induction compared to no induction, with a possible reduction in acute rejection with IL-2 RA compared to no induction. Overall there was no significant difference found for any comparison group for mortality, or adverse events including significant differences in renal function (Penninga et al. 2013).

3 Immunosuppression: Hypertension and Metabolic Disease

The use of Immunosuppression has led to the tremendous improvement in graft survival. However, immunosuppressants have been found to cause a variety of metabolic derangements including but not limited to: insulin resistance and diabetes, hyperlipidemia, hypertension, and weight gain after transplantation. This combination of metabolic risk factors may be associated with increased cardiovascular disease (Grundy et al. 2005). In addition many transplant recipients may have

many of these risk factors pre-transplant that are exacerbated by immunosuppression. These facts emphasize the need for rigorous follow-up and management of these risk factors post-transplant.

The most common immune suppressant regimens may include different combinations of these agents: Corticosteroids, Calcineurin inhibitors (CNIs), Mammalian Target of Rapamycin (mTOR) Inhibitors, Antimetabolite.

3.1 Corticosteroids

Corticosteroids have been an integral part of immunosuppression regimens since the beginnings of clinical transplantation (Bell et al. 1971). They are usually started at high doses and titrated off over a variable time frame ranging from 1 to 6 months. Steroids can lead to insulin resistance, hypertension, hyperlipidemia, and weight gain due to enhanced appetite. The steroid effect on glucose levels is dose dependent and generally improves as steroids are tapered off. Steroids stimulate insulin resistance by decreasing beta cell insulin production and increase in gluconeogenesis and a decrease in glucose utilization (Watt 2011). Steroids are also thought to cause hypertension due to sodium and water retention due to the mineralocorticoid effect and by vasoconstriction due to the glucocorticoid effect on smooth muscle (Goodwin et al. 2008). Long-term use of steroids is also associated with hyperlipidemia. Steroids can lead to an increase in both low-density lipoprotein (LDL) and high-density lipoprotein (HDL) levels and have a minimal effect on triglycerides. The mechanism by which LDL increase takes place is thought to involve the increased production of very low-density lipoprotein (VLDL) cholesterol and increased activity of 3-hydroxy-3-methyl-glutaryl-coenzyme A, and decreased LDL receptor function. The increase in HDL is attributed to the increase in lipoprotein lipase activity and a decrease in hepatic triglyceride lipase activity (Lau et al. 2010).

3.2 Calcineurin Inhibitors (CNIs)

CNIS are associated with hypertension, hyperlipidemia, and impaired glucose metabolism (Moien-Afshari et al. 2003). Tacrolimus (Tac) has a lower incidence of hypertension and hyperlipidemia when compared to cyclosporine (CsA). However Tac has been linked to higher levels of new onset diabetes and impaired glucose intolerance (Pham et al. 1996; Reichart et al. 1998; Eisen and Ross 2004).

CNIs have been thought to cause hyperlipidemia through decreasing bile acid synthesis from cholesterol and reducing cholesterol transport into the intestines leading to increased serum levels. In addition, cyclosporine can bind to LDL cholesterol receptor leading to the increase level of circulating LDL cholesterol (Muñoz 1995). The diabetogenic effects of cyclosporine and tacrolimus include toxic effects on pancreatic B cells which were more prominent with CsA, and inhibition of basal insulin secretin which is more prominent with TAC in the acute

phase. Taken together these effects result in reduced insulin synthesis, secretion, and increased insulin resistance (Øzbay et al. 2011).

The cause of hypertension secondary to CNIs is multifactorial. Systemic vasoconstriction combined with a decrease in glomerular filtration and enhanced sodium reabsorption in the renal tubules are thought to be the main culprits (Watt 2011). This vasoconstriction is thought to be related to the impaired balance of vasodilatory mediators (prostacyclin and nitric oxide) vs vasoconstricting mediators (endothelin, thromboxane A2, and the renin-angiotensin system) (Vaziri et al. 1998).

For those maintained on CsA considerations should be given when switching over to Tac if hyperlipidemia and hypertension are an issue. In addition, exposure to CNIs should be minimized to the lowest optimal level needed to maintain allograft to avoid the metabolic consequences discussed.

Because of all the potential metabolic side effects of both agents, efforts to minimize calcineurin inhibitor dosing are ideal. If the dominant issues are hyperlipidemia and hypertension, the conversion from cyclosporine to tacrolimus may result in improvements of these comorbidities.

3.3 Mammalian Target of Rapamycin (mTOR) Inhibitors: (Rapamycin/Sirolimus)

Sirolimus is a potent immunosuppressive drug capable of significantly reducing acute graft rejecting. However, hyperlipidemia is a major adverse event associated with Sirolimus. Sirolimus is thought to induce or exacerbate hyperlipidemia in a reversible and dose-dependent manner. It can increase total cholesterol and LDL with a much more potent effect on triglycerides. The mechanism leading to hypertriglyceridemia is thought to be multifactorial. Sirolimus can interfere with insulin-dependent adipocyte triglyceride storage. There is an increase in apolipoprotein B100 (associated with VLDL and LDL cholesterol), an increase in C III levels (which is a lipoprotein lipase inhibitor), and an increase in apolipoprotein C II (a lipoprotein lipase activator for triglyceride hydrolysis) (Morrisett et al. 2002).

Sirolimus also effects glucose metabolism and can cause glucose intolerance through unrestrained activation of hepatic gluconeogenesis. This can lead to the occurrence of a diabetes-like syndrome in patients (Houde et al. 2010).

3.4 Antimetabolites: MMF (CellCept)

Although very little data exist for the specific effects of MMF on metabolic comorbidities. Thus, this agent should be considered as an additional immunosuppressive agent that could allow dose reductions of the background immunosuppression (calcineurin or mTOR inhibitors) in patients with hypertension and metabolic disease.

Immunosuppression: hypertension and metabolic disease		
Immunosuppressant	Effect	Comments
Corticosteroids	Hyperlipidemia HTN Insulin resistance Weight gain	– With prolonged use: ↑LDL, ↑HDL, TG↔ – Salt, water retention and Vasoconstirction – Dose dependent, reversible with taper off – Enhanced appetite
Calcineurin inhibitors Tacrolimus (Tac) Cyclosporin (CsA)	Hyperlipidemia HTN Reduced insulin synthesis and increased resistance	– CsA > tac – CsA > tac – Tac > CsA
mTOR inhibitors Sirolimus	Hypertriglyceridemia Glucose intolerance and insulin resistance	
Antimetabolites MMF (CellCept)	No significant effect	

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