Principles of Immunotherapy

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Introduction

Immunotherapeutic intervention varies from immunomodulation, which adjusts the immune system back toward a state of homeostasis, to immunosuppression, which ablates specific compartments or pathways involved in the pathologic process. These approaches carry both benefit and risk. This chapter will discuss current and future principles of immunotherapeutic approaches.

Autoimmunity

Autoimmune disease results from failure of tolerance, the ability to discriminate between self and nonself. The immune system may then attack the individual's own cells and tissues. An inflammatory state may arise due to excessive activation of effector cells (resulting in a pro-inflammatory state) or insufficient regulatory cells leading to a loss of immune tolerance [1]. Several mechanisms work together to prevent autoimmunity. These mechanisms include central and peripheral tolerance, including T cell depletion, clonal anergy, and immune suppression provided by an important subpopulation of T regulatory (Treg) cells. These cells may carry either a CD4+ or CD8+ phenotype and include CD25+FoxP3+Tregs. Immunologic tolerance is controlled by this population of T cells [2]. Restoration of tolerance may be critical to the effective resolution of autoimmune disease processes (Fig. 2.1).

In addition to the loss of immune homeostatic balance in those with autoimmune conditions, genetic predisposition provides a further complex association. Multiple gene loci, most importantly the MHC/HLA haplotypes, are fundamental for the presentation of peptide antigens to T cells. Environmental variables such as geography, exposure, commensal microbiota, and infection also play a key role. Infections may activate self-reactive lymphocytes and lead to the development of autoimmune diseases in predisposed individuals.

Many autoimmune diseases follow a relapsing-remitting course, with periods of exacerbation followed by stability. This may relate to infection-triggered immune changes. The initiating response amplifies rapidly via activation of the

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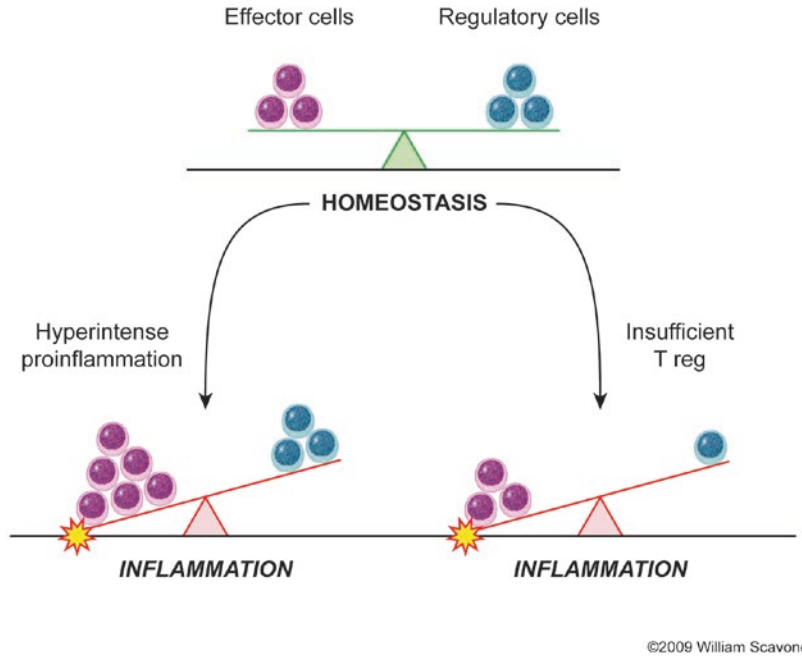

Fig. 2.1 Homeostatic balance of immune system. (Reprinted with permission from William Scavone)

innate immune system but is soon followed by a more target-specific response via the adaptive immune system. This includes antigen-specific T cells and antibody-producing B cells. Cytotoxic T cells and antibodies lead to efficient destruction of the invading microbe by eliciting specific inflammatory molecules, such as the interleukins that further activate the immune system and destroy the target in a variety of ways (including direct cell to target contact and oxidative molecules such as nitric oxide). Once the invading organism is eliminated the reduction in the immune response is rapid, limiting the damage to host tissue. Memory cells persist and provide the basis for secondary antigen-specific response. In autoimmune disorders, the tissue damage and immunological response does not completely subside, although clinical remissions are commonplace [3].

Clinical autoimmunity arises as a result of an altered balance between autoreactive effector cells and regulatory [1, 4]. The goal of treating autoimmune disease is to re-establish immune homeostasis and restore the balance between effector and regulatory T lymphocytes. Current immunotherapies are primarily used to intervene early and reduce epitope spread, induce and support the "quiescent" stage, and prevent future exacerbations.

The immune system may often seem overwhelming and too complex for the non-immunologist to fully understand, but there are recognized patterns to make organizing the information and concepts easier. The immune system is always trying to maintain balance, so for each action, there is an equal and opposite reaction. Cell lineage and generative lymphoid organs form a second pattern (Fig. 2.2).

T Cells

In T cell-mediated autoimmunity one of the most important players is the CD4+ T cell. Emerging from the thymus, naïve CD4+ cells differentiate into subtypes based on the cytokines they encounter in the periphery and/or within the CNS. Each CD4+ T cell subtype exhibits unique functions largely based on the cytokines they produce [5]. CD4+ T cells are both effector and regulatory. Effector CD4+ T cells can be categorized as either Th1 or Th2 T cells by their cytokine production. The signature cytokine for Th1 cells is interferon (IFN)-γ and for Th2 cells is IL-4 (Fig. 2.3). Upon encounter with antigen/MHC complexes, naive T cells become activated

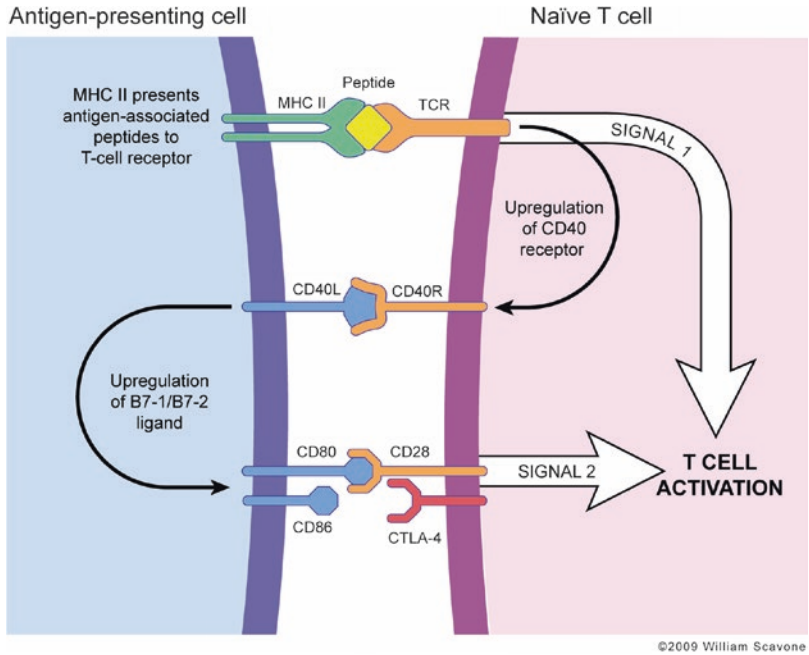

Fig. 2.2 Adaptive immune activation. Co-stimulation and T cell activation: full activation of T cells in the periphery is dependent on the recognition of co-stimulation factors on antigen-presenting cells (APCs) and completion of the two-signal activation. The first signal is comprised of antigen recognition: the APC presents MHC-associated antigenic peptides to the T cell receptor (TCR) on the naive T cell. Chemokines are released from the APC that react with the G-protein-coupled receptor (GPCR) on the T cell, increasing the affinity and avidity of the T cell/APC adhesion. Once the first signal is complete another set of molecules participate in increasing co-stimulatory signaling and secreting polarizing cytokines; for example, CD40 receptor is upregulated on the APC and engages with the constitutively expressed CD40 ligand on the T cell. The second signal is comprised of an upregulation of B7-1/B7-2 (CD80/CD86) ligand on the APC, following antigen recognition, that binds to the CD28 receptor on the T cell. Once the second signal is complete, the T cell is activated leading to clonal expansion and differentiation into effector functions. It is important to note that without the completion of the second signal the T cells become functionally inactive, anergic. (Reprinted with permission from William Scavone)

and can polarize into either a Th1 or Th2 cell. The process is influenced by a variety of factors, the most important of which is the cytokine milieu. The principal cytokines produced by antigen-presenting cells (APCs) for influencing Th1 cell polarization is IL-12, and for the TH2 it is IL-4 (Fig. 2.3). Once polarized, on the single-cell level the CD4+ Th1 and Th2 cells are committed and cannot revert back to a naive phenotype or convert to the other lineage. Using the early definition of T cell functions, IFN-γ facilitates macrophage activation and IL-4 facilitates the production of certain immunoglobulin subtypes. However, the lines between Th1 and Th2 functions have become blurred. IFN-γ is also required for the production of certain immunoglobulin (Ig) subtypes, and IL-4 can also be involved in macrophage activation [5]. The Igs induced by IL-4 serve specific functions, separating the activity of the two T cells. IL-4 is required for the production of IgG1 and IgE. IgE sensitizes mast cells, a consequence of which can be allergic reactions; IgG1 is involved in opsonization of pathogens. The IFN-γ-induced or classically activated macrophages produce nitric oxide (NO), which is pro-inflammatory and drives chronic inflammation and tissue injury. Other cytokines produced by Th2 cells that influence the immune response include IL-5, IL-6, and IL-13 (Fig. 2.3). Th1 T cells also produce IL-2, IL-15, granulocyte macrophage colony-stimulating factor (GM-CSF), tumor necrosis factor (TNF)-α, and other cytokines (Fig. 2.3). Like CD8+ T cells, Th1 cells also have the capac-

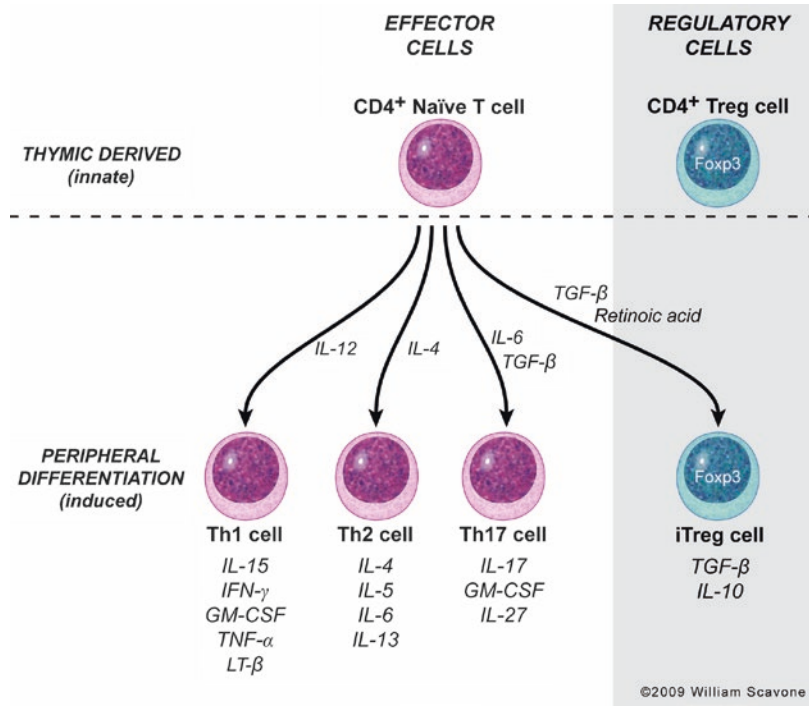

Fig. 2.3 Naive CD4+ lineage. Naive CD4+ cells emerge from the thymus and further differentiate into subtypes based on the cytokine microenvironment. Each subtype of CD4 T cells exhibits unique functions largely based on the cytokines that they produce. Treg cells are both thymic derived and induced in the periphery (iTreg). (Reprinted with permission from William Scavone)

ity to induce cytotoxicity of target cells by several different mechanisms. The immune response can be shaped by controlling the phenotype of the responding CD4 T cell [5].

Treg cells are essential in the everyday control of immune responses and maintaining peripheral tolerance [6, 7]. Two populations of T_{regs} control inflammation: natural (constitutive) Treg cells and induced Treg cells (iT_{reg}) (Fig. 2.3). Natural T_{reg} cells are a population of CD4+ lymphocytes residing in the thymus that express the interleukin (IL)-2 receptor CD25 and the transcription repression factor FoxP3. These cells constitute 5–12% of the entire CD4+ cell population and represent a very small proportion of the circulating WBC population. Specific populations of natural T_{reg} cells are generated principally by interaction with immature APCs in the periphery. They recognize major histocompatibility complex (MHC) molecules in association with autoantigens with high specificity. These natural T_{reg} cells are normally anergic but can be activated by exposure to antigens or to high concentrations of IL-2 released from activated TH1 cells. Induced T_{reg} cells are derived from either naïve CD8+ or CD4+ precursor cells in the thymus in response to the local antigen or cytokine environment. Three subpopulations of iT_{reg} cells can be distinguished on the basis of surface markers: CD8+ T_{reg} cells, TH3 cells, and TR1 cells. The latter two are derived from CD4+ precursors. In autoimmune disease, autoantigens can stimulate the differentiation of these iT_{reg} cells. iT_{reg} cells release cytokines such as IL-10 and TGF-β (Fig. 2.3) that suppress the activity of effector T cells as well as of APCs. Effector cells and APCs may be inhibited by direct contact with natural and induced T_{reg} cells and involve interactions of cell surface proteins. This helps prevent the development of hypersensitivity reactions of allergies, autoimmune disease, and promotes long-term graft tolerance. On the other hand, there may also be detrimental effects of inhibition of immune function by T_{reg} cells; it attenuates immunity to pathogens and reduces both immunological surveillance and prevention of tumorigenesis.

The best-studied T_{reg} cell to date is the Foxp3+ CD4+ T cell, a key regulatory molecule in the development and function of T_{reg} cells. FoxP3 is a

transcriptional repression factor of the Forkhead/winged box family. It is expressed by all functional T_{reg} cells except the TR1 class. Mutations in FoxP3 impair the development of T_{reg} cells in the thymus and are associated with inherited autoimmune diseases, such as Scurfy in the mouse and IPEX (an X-linked fatal autoimmune disorder) in humans [8, 9]. Seminal experiments have demonstrated that depletion of CD4+CD25+ suppressor cells results in the onset of systemic autoimmune disease in mice [10]. The defining influence of these cells in the control of autoimmunity was demonstrated in an experimental murine model. Foxp3 expressing cells were specifically depleted in adult mice, resulting in the development of rapidly fatal autoimmunity that involved a variety of host tissue beyond the lymphatic system [2]. Although the exact mechanisms by which T_{reg} cells regulate and suppress immune responses are not always clear, one method is through the production of the anti-inflammatory cytokine IL-10 [11]. IL-10 controls inflammation by regulating the expression of cytokines and molecules involved in antigen presentation. T_{reg} cells mediate peripheral tolerance by suppressing proliferation and cytokine production of autoreactive effector T cells that cause tissue damage and inflammation [12]. CD4 T cell population heterogeneity is essential for a properly functioning inflammatory response, and their differential production of cytokines is one method by which they exert their unique functions. As noted above, iT_{reg} can be derived from naïve CD8+ cells as well as CD4+ cells. The possibility that CD8+ T cells may also possess regulatory functions has received less attention, despite earlier studies [13]. CD8+ T cells can suppress the response of activated CD4+ cells. FoxP3 Treg cells inhibit the proliferation and cytokine production by both Th1 and Th2 cells and may suppress B cells [14].

NK Cells

Natural killer (NK) cells are a subset of bone marrow-derived lymphocytes, distinct from B and T cells, that function in innate response to kill microbe-infected cells and to activate phagocytes by secreting IFN-γ; they enhance the adaptive response against infectious agents [15]. NK cells do not express clonally distributed antigen receptors such as Ig or TCRs. Their activation is regulated by a combination of stimulatory and inhibitory cell surface receptors. The inhibitory cell surface receptors are responsible for recognizing self-MHC molecules [15]. The ability of NK cells to protect against infections is enhanced by IL-12 produced by macrophages, as well as antibody-mediated targeting. NK cells and other leukocytes may bind to antibody-coated cells and destroy them by opsonization. NK cells express an Fc receptor, FcγRIII (CD16), that binds to IgG antibody arrays attached to a cell [15]. As a result, NK cells are activated and kill the opsonized target, via antibody-dependent cellular cytotoxicity (ADCC). Although NK cell-mediated ADCC is not as important as phagocytosis of microbes in defense against most bacterial and viral infections [15], in autoimmunity the connection between infections and initiation/amplification of the aberrant immune response is key. NK cells play opposing roles in autoimmunity, as they function as both regulators and inducers of autoimmune diseases, dependent on the cytokine milieu and cell-cell interactions. NK cells comprise about 10% of the lymphocytes in the blood and peripheral organs.

IL-15 appears to play pivotal roles in the differentiation of NK cells from their progenitors and their survival and activation. CD56bright NK cells are an important NK cell subset that exerts immunoregulatory effects [16]. In vivo, blockade of the human IL-2R by a monoclonal antibody (daclizumab) has been used for immunosuppression in transplantation, to treat leukemia and autoimmune diseases. In one study, in uveitis patients, administration of a humanized IL-2R blocking mAb induced a 4- to 20-fold expansion of CD56bright regulatory NK cells. The induced CD56bright regulatory NK cells from patients exhibited similar phenotype to naturally occurring CD56bright cells. Patients with active uveitis had a significantly lower level of CD56bright NK cells compared with normal donors. In addition, the induced CD56bright cells, but not CD56dim

cells, could secrete large amounts of immunosuppressive cytokine IL-10. This suggests that the induction of the CD56bright cells might lead to the remission of active uveitis [17]. This observation may have implications for IL-2R blockade therapy and for the potential role of CD56bright regulatory NK cells in autoimmune diseases. By blocking the IL-2Rα chain the mAb can limit T cell expansion and direct the co-stimulated cell toward NK production (CD56bright) through the heterodimer IL2Rβ, inducing IL-15. Antibodies to IL-2Rα do not inhibit the action of IL-15 [18]. The IL-15 receptor includes IL-2/15R and γc subunits, which are shared with IL-2 and an IL-15-specific receptor subunit, IL-15R [18]. The induced expansion of NK cells produced similar phenotype and function as naturally occurring NK cells and correlated highly to the reduction of inflammatory activity in human and animal studies.

NKT Cells

Natural killer T (NKT) cells share characteristics of both T and NK cells and play a regulatory role in autoimmunity. NKT cells are thymically derived innate lymphocytes that express the TCR and receptors of the NK lineage, NK1.1. The TCR on the majority of the NKT cells expresses an invariant Va-Ja combination that translates into Va14 Ja281 (also called Ja18) in the mouse and Va24 JaQ in humans [19]. NKT cells recognize glycolipids, such as α-galactosylceramide (α-GalCer), presented by the CD1d molecule on APCs [20, 21]. Unlike the classical MHC molecule that presents protein to lymphocytes, the CD1d molecule presents glycolipids to the TCR on the NKT cell [22]. Because of TCR chain characteristics on classical NKT cells, they are also called invariant (i)NKT cells [19]. Invariant NKT cells (iNKTs) are regulatory T lymphocytes that are CD1d reactive with an invariant TCRα chain, Vα24-JαQVβ11 [21]. The regulatory function of iNKT cells is related to their rapid and diverse secretion of cytokines like IFN-γ, IL-4, IL-5, and IL-10 upon TCR stimulation. iNKTs play a dual role in the modulation of T cell-mediated immunity. They provide frontline defense against parasites, bacteria, and viruses and induce tolerance for the prevention of autoimmune diseases (similar to that of classical T_{regs}). Balancing the two functions of adjuvant and regulation is related to the microenvironment, either to build an effective inflammatory immune response (upregulation of IL-12/IL-23 by APC or effector cells) or prevent autoimmunity with regulation/counter-regulation (upregulation of CD1d or IL-10 by APCs or effector cells). In EAE, it was noted that the lipid structure of the CD1 ligands influences the duration of interaction between APCs and iNKT cells and thus the cytokine secretion by the activated iNKT cell. A shortened glycolipid and TCR contact time produced TH2 cytokine profile, while a longer glycolipid and TCR contact time resulted in a pronounced TH1 cytokine profile of iNKT cells [19]. Concerted interactions between iNKT cells and CD1d+ cells, DCs, macrophages, and B cells are involved in rendering autoreactive T cells unresponsive [19]. A primary goal in the treatment of autoimmune disorders is to find a therapeutic regime that inhibits reactive T cells while improving regulatory cell function. iNKT cells represent an important cellular bridge between the innate and adaptive arms of the immune system.

B Cells

The role of B cells in normal immunity is well understood. The role of B cells is less clear in autoimmune diseases and historically associated with antibody production, the antibody-dependent role. Lymphocytes are the main immune cells. As discussed earlier, T lymphocytes dictate cell-mediated immunity. B lymphocytes are responsible for humoral immunity, the host defense mediated by secreted antibodies that protect against extracellular microbes and their toxins [15]. Humoral immunity is important to prevent infection. Generation of the mature B cell pool involves stepwise development of hematopoietic stem cells into pro-B cells, which mature into pre-B cells and then

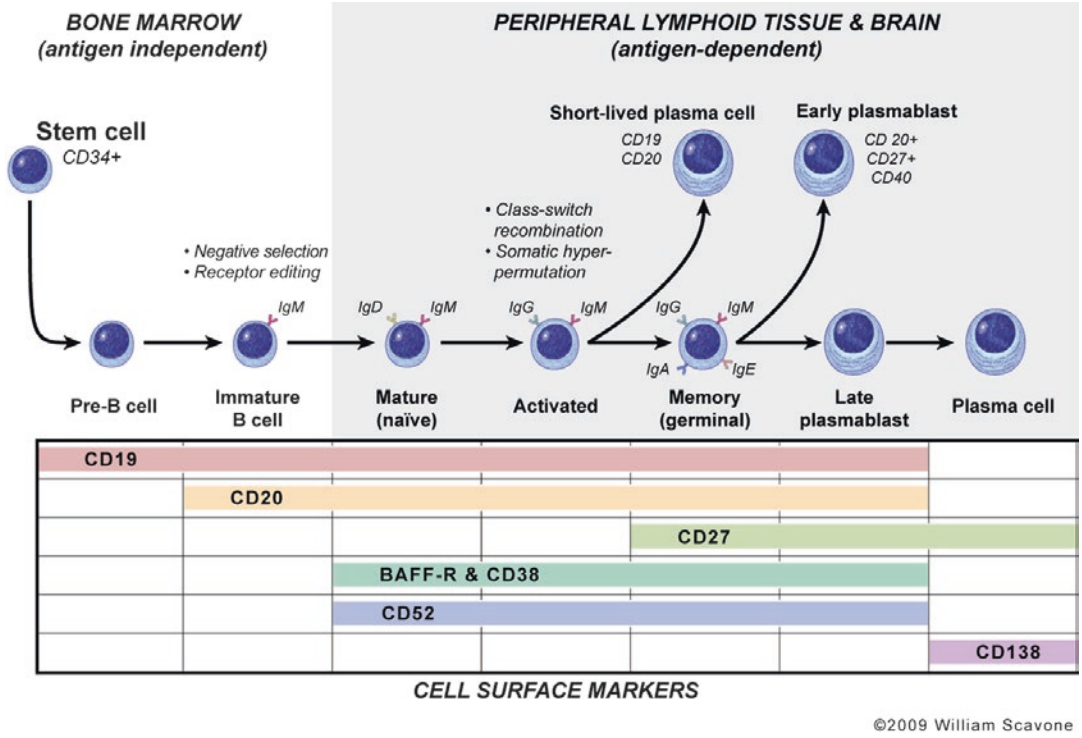

Fig. 2.4 B cell maturation and humoral immune response. The maturation of B lymphocytes proceeds through sequential steps. Many of which occur within the bone marrow. There are receptor editing and negative selection prior to maturation. Once mature, the naive IgM+IgD+ B cell is able to recognize antigen and undergo activation upon engagement with T lymphocytes and stimuli within the microenvironment. The activated, antigen-specific, effector cells can undergo class switching and affinity maturation, improving the capacity to identify and bind to an identified antigen. The expression of cell surface receptors is important to the understanding of B cell therapeutic targets for autoimmunity. (Reprinted with permission from William Scavone)

immature B cells [15, 23] (Fig. 2.4). Immature B cells are then exported to the periphery as transitional B cells, which undergo further selection and development. When mature IgM+IgD+ B cells encounter T cell-dependent antigen (Ag), they differentiate into high-affinity effector cells, namely, memory B cells and immunoglobulin (Ig)-secreting cells (plasma cells) within the secondary lymphoid tissue of germinal centers [15, 23] (Fig. 2.4). Mature B cells are responsible for the generation of humoral immunity and long-lived serological memory. The coordinated differentiation of B cells at these different stages of development and maturation is influenced by multiple factors, such as stromal cells and cytokines provided within the bone marrow environment, Ag exposure, and interactions between B cells, Ig-specific T cells, and dendritic cells (DC) in the periphery [15, 24, 25]. Accumulating evidence strongly supports an increased involvement of B cells in autoimmune neurological diseases, with noted antibody-dependent and antibody-independent roles.

B cell development is complex and a multiple-step process. Differentiation of mature B cells into effector cells must be strictly regulated to ensure sufficient specific humoral immunity while simultaneously avoiding the production of autoantibodies. Receptor-ligand pairs of the tumor necrosis factor receptor (TNF-R/TNF) superfamily play critical roles in humoral immunity by regulating activated B cell responses [26]. Two members of the TNF family, B cell activating factor (BAFF) and a proliferation-inducing ligand (APRIL), have been identified in recent years as crucial factors for B cell survival,

differentiation, germinal center formation, and antibody production [27]. BAFF binds three receptors, which all belong to the TNF-R superfamily—BAFF receptor (BAFF-R) [28], transmembrane activator and calcium modulator and cyclophilin ligand interactor (TACI), and B cell maturation antigen (BCMA) [29, 30]; the latter two receptors also bind APRIL [27]. BAFF is predominately produced by myeloid cells such as macrophages, monocytes, dendritic cells, and astrocytes [31, 32] and neutrophils [33]. However, production can be further induced by cytokines (INF-γ and IL-10) [32, 34]. Pathogen-associated molecular pattern (PAMP) molecules and toll-like receptors (TLR) can also induce production of BAFF in B cells, in response to microbial components such as peptidoglycan, CpG dsDNA, and lipopolysaccharide (LPS) when they are within contact [35, 36]. BAFF is required for late B cell development and maintenance of B cell homeostasis. Normal human B cells first express BAFF receptors at the transitional stage of development and remain capable of receiving BAFF-dependent signals at least until they terminally differentiate into plasma cells (PC) (Fig. 2.4). Dysregulation of BAFF has been observed in patients with many systemic autoimmune diseases. The serum levels of BAFF are notably increased in these patients and correlated with the severity of their symptoms [37–41]. It is speculated that BAFF protects self-reactive B cells from deletion by modifying the expression of pro- and anti-apoptotic molecules; it reduces the pro-apoptotic molecules while increasing the anti-apoptotic molecules [24, 26] and impairs B cell self-tolerance. Normally, BAFF provides survival signals for B cells involved in immune defenses against infection. Elevated BAFF levels are involved in the survival of self-reactive B cells and autoimmune diseases. BAFF does not affect the central self-tolerance of B cells during their early development in bone marrow, but influences the peripheral self-tolerance of B cells, especially in later transitional stages of B cell development (Fig. 2.4) [26, 42]. The relationship between BAFF and toll-like receptor (TLR) signaling is strong in mouse models of autoimmunity [36] and therefore another potential area of therapeutic opportunity, as TLR signaling is also implicated in the pathogenesis of human autoimmune diseases [26]. Antagonists of BAFF are promising therapeutic agents to treat autoimmune diseases [26, 27].

Many organ-specific autoimmune diseases in humans are believed to be caused by T cells. Antibodies that cause disease are most often autoantibodies against self-antigens and less commonly are specific for foreign antigens. Autoantibodies may bind to self-antigens in tissues or they may form immune complexes with circulating self-antigens [15], such as in myasthenia gravis (MG). The contribution of activated B cells has traditionally been viewed as a secondary consequence of the breakdown of T cell tolerance. In certain neurological diseases, including myasthenia gravis and specific neuropathies, autoantibodies are pathogenic and exert a direct effect on self-antigens either by functioning as neutralizing antibodies or by activating and fixing complement on the targeted tissues (Fig. 2.5a) [27]. Normally the complement system helps eliminate microbes during innate and adaptive immune responses. Opsonization is probably the most important function of complement activation. However, during the membrane attack small peptide fragments are produced by proteolysis. These fragments are chemotactic for neutrophils and stimulate the release of inflammatory mediators from various leukocytes. Neutrophils also act on endothelium to enhance the movement of leukocytes and plasma proteins into affected tissues to eliminate microbes. In normal individuals, B cells are tightly controlled and prevented from making autoantibodies, perhaps via their interaction with T_{regs}. In autoimmune disorders, this process of activating and fixing complement by autoantibodies leads to activation of ADCC (Fig. 2.5a) [27]. In ADCC, NK cells and other leukocytes may bind to antibody-coated cells and destroy them.

Another mechanism of B cell involvement in autoimmune disorders involves the presentation of antigenic peptides, with clonal expansion of either autoreactive or regulatory T cells (Fig. 2.5b) [27, 43–45]. Divergence of T cell phe-

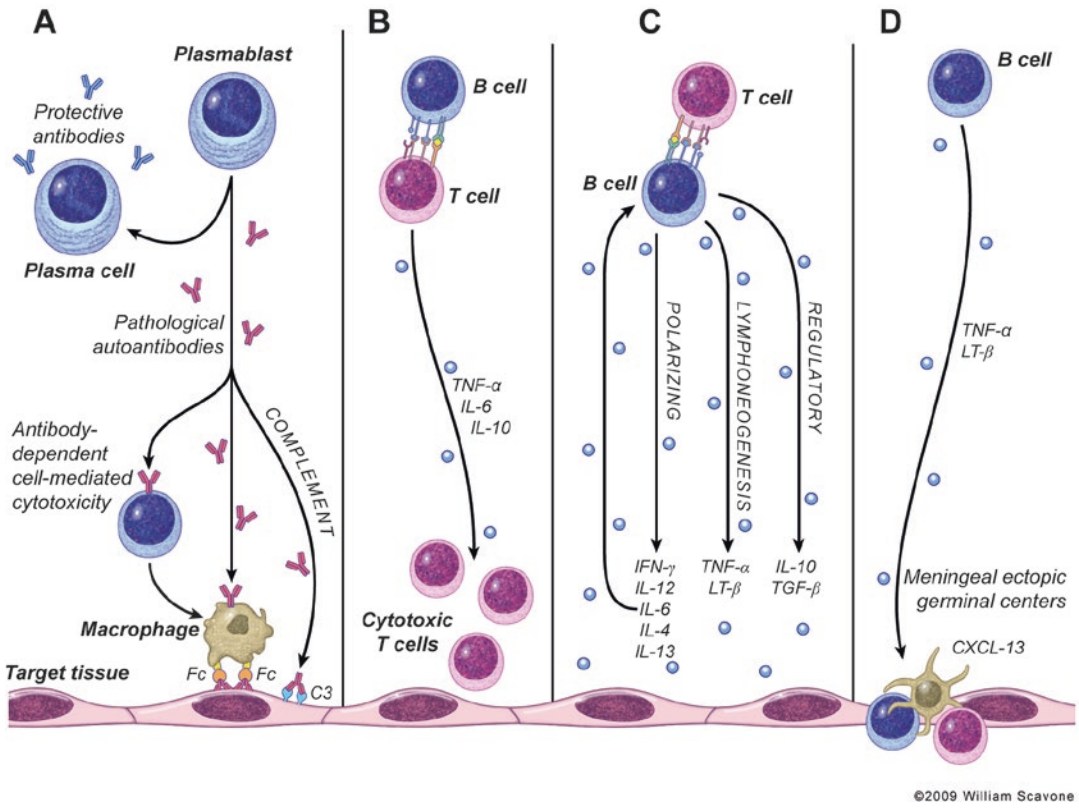

Fig. 2.5 B cell functions in autoimmunity. (**a**) Antibody-producing cells-plasma cells (**b**). Antigen-presenting cells (autoreactive T cells with a specific antigen; regulatory with low levels of nonspecific antigen) (**c**). Cytokine-producing cells; regulatory (B cell activation with isolated CD40 stimulation), polarizing (B cell activation with dual stimulation of BCR and CD40), lymphoneogenesis (memory B cells primarily produce pro-inflammatory cytokines, TNFα/LT following dual stimulation of BCR and CD40) (**d**). Development of tertiary/ectopic germinal centers. (Reprinted with permission from William Scavone)

notypes and functions relates once again to the environment and specificity of antigens. B cells present specific antigens to cognate CD4+ T cells with extremely high efficiency to drive autoreactivity, so that they obtain help from CD4+ T cells for the production of high-affinity antibodies. Nonspecific antigens derived from low levels of endogenous proteins are also presented by B cells, but the outcome of presentation of nonspecific antigens is T cell tolerance [43]. B cells are 100–1000 times more potent in antigen presentation than other postulated APCs, including dendritic cells [27, 46, 47]. B lymphocytes that bind protein antigens by their specific antigen receptors endocytose these antigens, process them in endosomal vesicles, and display MHC II-associated peptides for recognition. They are also effective at presenting low concentrations of antigen. The membrane Ig of the B cell is a high-affinity receptor that specifically binds a particular antigen, even when the extracellular concentration of the antigen is very low [15]. Affinity maturation is in response to an antigen and increases with prolonged or repeated exposure. In addition to presenting antigen via MHCII, B cells also express co-stimulatory factors (such as B7) that activate, via two-signal co-stimulation, the autoreactive T lymphocyte. This, in turn, activates T cells by expressing CD40 ligand and secreting cytokines. This promotes clonal expansion, proliferation, and differentiation. As this co-activation between B cell (APC) and T cell occurs, heavy chain class switching and affinity maturation are also stimulated, demonstrating

further that B cells play an important role in magnifying and sustaining the T cell response. Although the antigens are unknown, modulating/suppressing B cells and the ensuing co-stimulation of T cells may contribute to the treatment effects noted with early treatment of many neurological autoimmune disorders.

Cytokine-producing B cells influence the initiation of immune responses and regulate T cell responses. As noted in Fig. 2.5c, another antibody-independent function of B cells is the production of a diverse array of cytokines, including regulatory (IL-10, TGFβ), polarizing (IL-4, IL-13, IFN-γ, IL-12), and lymphoid tissue-organizing cytokines (TNFα, LTβ) [47, 48]. B cell-derived cytokines are produced and dictated by the balance of stimulatory signals via the B cell receptor (BCR) and CD40 [49]. CD40 is constitutively expressed on all B cells [50] and therefore B cells are capable of activation via BCR/CD40 ligation or singularly with CD40L via local immune responsive T cells [49]. Cytokines produced by B cells, including IL-6, play important roles in regulating autoimmune responses. IL-6 produced by activated (BCR/CD40 stimulated) B cells functions in an autocrine fashion. It induces differentiation of IL-6 receptor (IL-6R) expressing B cells into antibody-secreting plasma cells and enhances the long-term survival of the IL6-R+ plasma cells [47]. In normal B cells the IL-6/IL-6R autocrine loop is tightly regulated. Dysregulation of B cell-derived IL-6 has been suggested to contribute to the formation of autoantibodies and development and magnification of autoimmune disorders [47, 49]. IL-10 is a suppressive cytokine produced by normal B cells and B cells associated with autoimmune disorders. IL-10-producing B cells, in EAE, have the ability to downregulate the ongoing type 1 autoimmune response [51, 52] and suppress the expansion of autoimmune type 1 cells [53]. Duddy and colleagues demonstrated that naïve (CD19+CD27−) and memory (CD19+CD27+) human B cells express distinct profiles of effector cytokines and reconfirmed earlier findings of context-dependent cytokine production of IL-10 and TNFα/LT [2]. Regulatory B cells control active CNS demyelination in a murine EAE model [54]. Naïve B cells (CD19+CD27−) almost exclusively produce IL-10, specifically after B cell activation with isolated CD40 stimulation ex vivo [2, 49]. As a well-established regulatory cytokine that suppresses APC and T cell activation, B cell IL-10 likely decreases inappropriate immune responses by limiting undesirable polyclonal expansion and inducing apoptosis [49]. Memory B cells (CD19+Cd27+) primarily produce pro-inflammatory cytokines, TNFα/LT following dual stimulation of BCR and CD40 [2, 49]. It is important to remember the homeostatic function of the immune system; IL-10-producing B cells may ameliorate T cell-mediated autoimmune disease, while activated B cells are proficient producers of inflammatory cytokines, such as lymphotoxin (LT) and TNFα (Fig. 2.5d). Current and future therapeutics are focused on selective B cell depletion (anti-CD20 mAb) and chemoablative techniques (anti-CD52 mAb, autologous stem cell therapy) [2].

Lymphotoxins and TNFα produced by B cells are responsible for organizing secondary and tertiary/ectopic lymphoid structures (Fig. 2.5d) [27] in autoimmune disorders. Ectopic lymphoid structures could represent a critical step in sustaining humoral autoimmunity and disease exacerbation in neurological autoimmune disorders [55]. In a healthy immune response, peripheral lymphoid organs are organized to concentrate antigen, APCs, and lymphocytes in a way that optimizes interactions among the cells and produces an appropriate adaptive response. An example of this organization would be in lymph nodes (LNs), specialized organs for trapping antigen from local tissue supplied by lymphatic vessels. LNs can be divided into three regions: cortex, paracortex, and medulla [15]. Naïve mature B cells are drawn into developing LNs by expression of the chemokine CXCL13. These B cells are then organized into follicles containing follicular dendritic cells (FDCs), located in the cortex of LNs, surrounded by T lymphocytes within the paracortex containing dendritic cells (DCs). The organization of the T and B cells adjacent to one another enables the two cells to migrate toward each other and interact to help B

cells differentiate into antibody-producing cells. Normally affinity maturation occurs in the germinal centers of lymphoid follicles, as a result of somatic hypermutation of the Ig genes [15]. In autoimmune disorders, LT produced by B cells facilitates the development of tertiary structures, referred to as lymphoid neogenesis, occurring in the intermeningeal spaces of patients with MS, in the thymus of myasthenia gravis patients, and in the target organs associated with RA, Sjogren's, and thyroiditis [15, 47]. Ectopic germinal centers of the thymus have also been found to develop preferentially in patients with early onset myasthenia gravis (EOMG) [56, 57]. In other autoimmune diseases, it has been demonstrated that ectopic follicles are found in tissues with the highest degree of inflammation, indicating that formation of ectopic lymphoid tissue requires a strong immune activation via autoimmune dysregulation and/or infectious stimulus (viral/bacterial) that results in a persistent inflammatory microenvironment [58, 59]. Formation of ectopic lymphoid tissue is viewed as part of an adaptive response against infection. It may also have the potential to support autoimmunity through expansion and activation of autoreactive B and T lymphocytes and further destruction of tissue [68]. Therapeutic targets (possibly B cell depletion, chemokine antagonists, or LTβR-Ig) should be focused on prevention or eradication of such tertiary lymphoid structures nested within the CNS and other target organs of autoimmunity.

Trafficking Molecules

The central nervous system (CNS) is characterized by an immune-specialized environment as a result of limited lymphatic drainage, resident DCs, and MHC expression [15, 60]. Under normal conditions, the CNS strictly controls immunosurveillance, localized to the perivascular and subarachnoid spaces, as it is crucial for host defense [60]. Often the blood-brain barrier (BBB) is the only site of leukocyte transmigration. There are three potential sites for leukocytes to enter into the CNS: the BBB, the blood-CSF barrier (BCSFB), and the blood-spinal cord barrier (BSpCB) [60, 61]. The remaining discussion will focus on the BBB, which should be thought to include both capillary and postcapillary venules (they show equal restriction of molecules, with no differential characteristics) [60]. Slight differences between BBB meningeal and parenchymal microvessels have been identified. The meningeal microvessels lack astrocytic ensheathment [62], while the parenchymal microvessels lack P-selectin [63]. The choroid plexus epithelium establishes the brain-CSF barrier (BCSFB). Data suggests that lymphocytes enter the CSF across the BCSFB during normal immunosurveillance to monitor the subarachnoid space. They retain the capacity to initiate a local immune reaction if needed or return to secondary lymphoid organs, via CCR7 and L-selectin [63]. Ventricular and lumbar CSF from healthy patients is uniformly composed of CD4+ central memory T cells [64]. What guides autoreactive leukocytes (lymphocytes, macrophages, monocytes, eosinophils, neutrophils) into the CNS in neuroimmune inflammation disorders is still unclear. Whether antigen presentation takes place in the cervical or lumbar lymph nodes, as both are specific lymphatic drainage sites for CNS solutes (molecular mimicry) and antigens (neuro-specific antigens) [65], is not yet clarified. There are chemokine gradients between brain parenchyma and circulation that could be initiated by a viral or bacterial infection that would then trigger TLRs in innate immune cells of the brain (microglia and astrocytes) [66]. Could prolonged inflammation and/or specific BBB transmigration thru postcapillary venules give way to ectopic germinal center formation and amplification of the disease process? Understanding the mechanisms of leukocyte trafficking into the brain might provide insight into how to modulate pathologic immune responses with specific therapeutic targets.

Leukocyte transmigration is governed by chemoattractant cytokines, chemokines, and adhesion molecules and is a multistep well-orchestrated response to injury and inflammation (Fig. 2.6). It requires specific adhesion molecules (AMs), selectins, to make transient contact with the endothelium cells. Autoreactive leukocytes loosely tether and roll along the endothelial cells

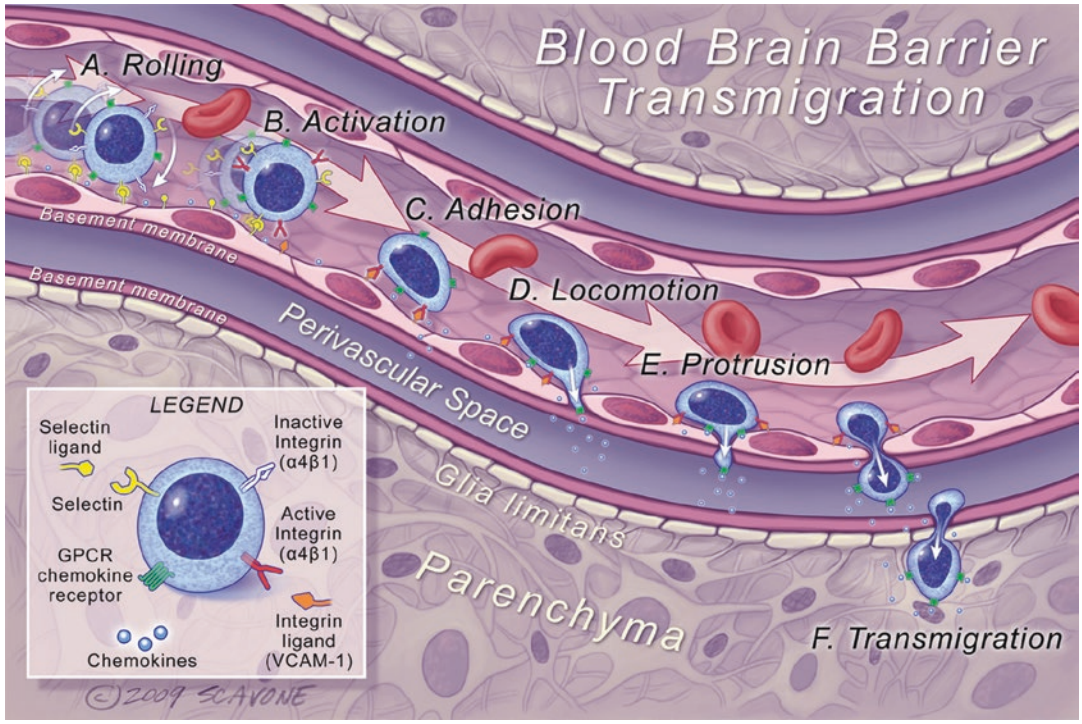

Fig. 2.6 Blood-brain barrier transmigration. Multistep recruitment of leukocytes across the blood and CSF barriers in the inflamed brain. (**a**) Rolling: Autoreactive leukocytes loosely tether and roll along the endothelial cells due to the binding of selectins and associated ligands. The shear forces of the blood flow continue the autoreactive leukocyte in a rolling motion while sensing activating factors. (**b**) Activation: Once the rolling leukocyte slows in velocity, it reacts to chemokines on the endothelial surface via G-protein-coupled receptor, resulting in activation and conformational changes of integrins on the leukocyte surface. (**c**) Adhesion: Activation leads to an increased affinity and avidity for endothelial ligands and arrest of the leukocyte rolling motion. Only activated leukocytes are able to mediate firm adhesion. (**d**) Locomotion: Arrested leukocytes move across the endothelial surface until the tight junctions of the endothelium, interendothelial junctions, are identified. (**e**) Protrusion: Activated leukocytes extend protrusions through the tight junctions sensing chemokines that serve as guides. (**f**) Transmigration: Diapedesis of leukocytes through the endothelial barrier between the endothelial basement membrane and the basement membrane of the glia limitans within the perivascular space. Matrix metalloproteinases (MMPs) facilitate the leukocytes migrating both basement membranes and the glia limitans, providing entry into the parenchyma. (Reprinted with permission from William Scavone)

due to the low-affinity binding of selectins and associated ligands (Fig. 2.6a) [60, 61]. There are three types of selectins: L-selectin is expressed on most circulating leukocytes, while P- and E-selectin expressions are inducible on endothelial cells involved in acute and chronic inflammatory processes. The shear forces of the blood flow continue the autoreactive leukocyte in a rolling motion, while it senses activating factors on the endothelial surface [60, 61]. Luminal chemokines are immobilized on endothelial surfaces to trigger activation of integrins from circulating leukocytes (Fig. 2.6a) [61].

Once the rolling leukocyte slows in velocity, it reacts to chemokines on the endothelial surface via G-protein-coupled receptor, resulting in activation and conformational changes of integrins on the leukocyte surface (Fig. 2.6b). Integrins are a large family of αβ heterodimeric transmembrane proteins that provide a physical linkage, mediating cell-cell and cell-extracellular matrix interactions, and help to regulate cell behavior through discrete regulatory cues [15]. Upregulated integrins on the autoaggressive leukocytes include P-selectin glycoprotein ligand-1 (PSGP-1) and very late antigen-4 (VLA-4)/α-4 integrin

(α4β7). G-protein-dependent activation leads to secure lymphocyte fixation, due to increased affinity and avidity of integrins for endothelial ligands vascular cell adhesion molecule-1 (VCAM-1) and intracellular adhesion molecule-1 (ICAM-1) (Fig. 2.6c). Newly identified adhesion molecules, junctional adhesion molecule-A (JAM-A) and platelet-endothelial cell adhesion molecule-1 (PECAM-1), are involved in the permeability and transmigration of the BBB [60]. They may be future therapeutic targets.

Only activated leukocytes mediate firm adhesion and arrest of rolling. They then travel across endothelial surfaces until they identify interendothelial tight junctions (Fig. 2.6d) [67]. Activated leukocytes extend protrusions through the tight junctions in response to chemokines (Fig. 2.6e) [61]. Chemokines are a large family of low-molecular-weight chemotactic cytokines that direct cells to specific sites of inflammation or injury and play an important role in leukocyte homing [68]. Chemokines secreted by lymph node cells attract B cells to germinal centers, DCs and T cells to T cell areas. The chemokine family is comprised of approximately 50 molecules and 20 receptors [68, 69]. The chemokine ligand superfamily is divided into subgroups, the largest being CC chemokines (28 members), CXC chemokines (16 members), and CX3C chemokines (1 member) [68, 70]. Subgroup members are functionally related and signal to corresponding families of chemokine G-protein-coupled receptors (GPCRs). Most of the receptors bind several different chemokines, and many chemokines bind different receptors. Chemokine receptors are localized to various cell types, direct adaptive immune responses, and contribute to the pathogenesis of many diseases. In the CNS, specific chemokine receptors have been detected on microglia, astrocytes, oligodendrocytes, neurons, and brain microvasculature [68]. Chemokines are implicated in many autoimmune disorders as they regulate a multitude of effector cells by governing their departure from the bloodstream into tissues, their migration through lesions, and their effector functions. Assigning roles to individual receptors is critical to the identification of relevant therapeutic targets.

Transmigration (diapedesis) occurs as leukocytes extravagate thru the endothelial barrier, between the endothelial basement membrane and the basement membrane of the glia limitans within the perivascular space (Fig. 2.6f). Activated cells (including monocytes, macrophages, T lymphocytes, neutrophils, endothelial cells, microglia, astrocytes, oligodendrocytes) secrete matrix metalloproteinases (MMPs). MMPs are enzymes that digest various collagen components of the extracellular matrix and basement membrane [71]. Tissue inhibitor of metalloproteinases (TIMP) controls the activity of MMPs. MMPs in coordination with TIMP facilitate the final step of leukocytes migrating the basement membrane and glia limitans, providing entry into the parenchyma [61]. There are many immunological targets to halt leukocyte trafficking into the parenchyma including, but not limited to, G-protein-coupled receptor, adhesion molecules, chemokines, and MMP/TIMP.

S1P1

A newer therapeutic paradigm to affect leukocyte transmigration involves blocking leukocyte lymphoid and thymic egress, thru sphingosine 1-phosphate (S1P). S1P is an important signaling molecule produced inside cells by sphingosine kinase-driven phosphorylation [72]. Once the S1P cells are transported and externalized into blood and interstitial fluids, they actively engage with associated G-PCRs, regulated by cellular activation, on a multitude of cells. Both sphingolipid metabolites, S1P and ceramide, have been identified as critical regulators of cell survival and death [73]. S1P is associated with decreased apoptosis, while ceramide conversely is associated with pro-apoptosis. Not only do these two sphingolipid metabolites exert opposing roles, but they are also interconvertible. This suggests the dynamic ratio between S1P and ceramide is responsible for cell fate [74], and ultimately health or disease, in a wide distribution of systems. S1P receptors 1–5 are ubiquitously expressed, but show differential cell association and physiological action [72]. In the context of neurological autoimmunity, S1P1 normally trans-

duces S1P effects on lymph node (LN) egress and tissue migration of naive lymphocytes, S1P4 has been detected primarily in the immune compartments and leukocytes [75], and it has been postulated that S1P4 may participate in cytokine production by T lymphocytes [76]; S1P5 is expressed primarily in the CNS white matter tracts, specifically in the oligodendrocytes [77]. S1P1 receptor regulates the mobilization of NKT cells to inflammation within the periphery [78]. S1P1 agonist prevents lymphocyte egress from secondary lymphoid tissues, resulting in a reduction of peripheral lymphocytes and therefore limiting potential recirculation into the CNS. Small molecules proligand (agonists) and modulators for sphingosine 1-phosphate receptor (S1P), S1P receptor agonists and modulators, are approved or being developed for the treatment of MS. The T1IFNb protein, CD69, also impairs the function of S1P1 in a similar function [79]. S1P is a clear therapeutic target for many serious medical conditions such as cancer, inflammation, and immune-mediated disorders such as MS.

Dendritic Cells

DCs are bone marrow-derived cells (HPCs) (Fig. 2.7), found in epithelia and most organs, morphologically characterized by thin membra-

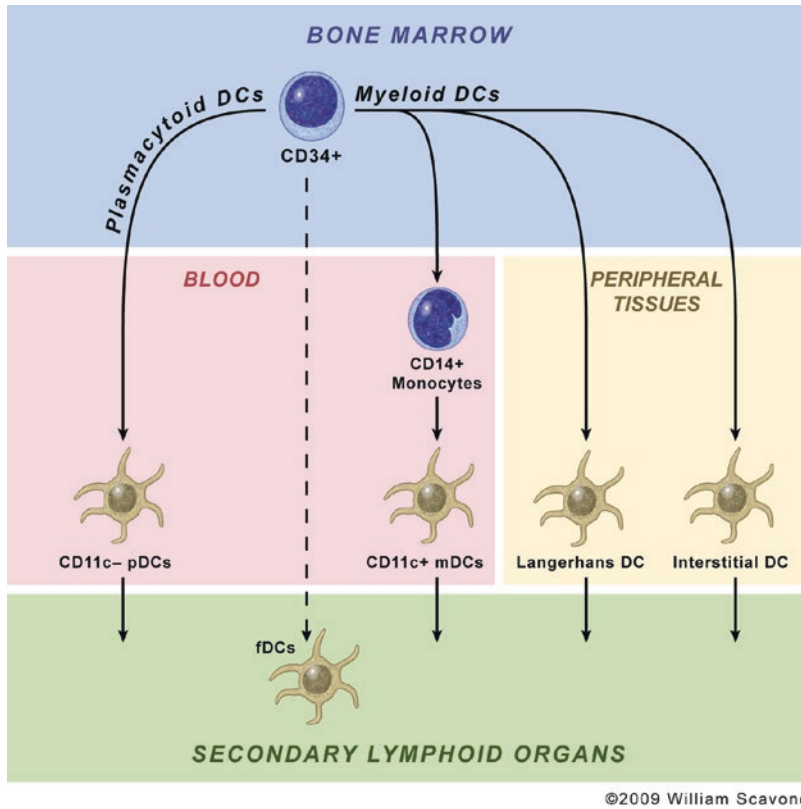

Fig. 2.7 Dendritic cell lineage and subtypes. Two main pathways of dendritic cells (DCs) originate from bone marrow hematopoietic progenitor cells (HPCs), into myeloid DC (mDCs) and plasmacytoid DC (pDCs). Resident DC is mature and found in the secondary lymphoid tissues. Follicular DC (fDC) is unique and found within the germinal centers of lymph nodes with the primary role of presenting antigen to B cells, not T cells. pDC exists in the blood/circulatory compartment. mDCs exist in the peripheral tissues, blood, and secondary lymphoid compartments. Within the peripheral tissues, there are two additional subtypes: interstitial DC (intDC), located within the dermis and responsible for humoral immunity, and Langerhans DC (LC), located within the epidermis and responsible for cell-mediated immunity. (Reprinted with permission from William Scavone)

nous projections, dendrites. DCs are specialized to capture and process antigens, to present their peptides to lymphocyte. They are found in all peripheral tissues, blood/circulatory system, and lymphoid organs [4, 80]. DCs play a pivotal role in orchestrating the immune response. The activation status and cytokine secretion profile of DCs control both activation and tolerization of immune responses against self and nonself antigens. They function as "professional" APCs for naïve T lymphocytes and are important for the initiation of the adaptive immune response to protein antigens [15]. Integral to specific autoimmune diseases is an imbalance in the production of a particular cytokine (i.e., rheumatoid arthritis, TNFα; systemic lupus erythematosus (SLE), T1IFN; MS, IL-12/23, IL-17) that are dependent upon DC interactions. In MS, it is well known that the cytokine profiles of CD4+ T lymphocytes are dictated by the ability of APCs (such as DCs) to secrete either IL-12/IL-23, for a Th1 response, or the combination of TGFβ and IL-6, for a Th17 response. In addition, DCs secreting IL-10 have been shown to induce IL-10-producing Tregs [81, 82]. The immune system is a dynamic system of cytokine vectors. Equilibrium maintains health and protective immunity, while a predominant skewing leads to autoimmunity and immunopathology. DC maturation and subsets play a critical role in stimulating immune responses as well as maintaining tolerance. This understanding has led to the potential of DCs as a distinct therapeutic target for various inflammatory and autoimmune diseases [4].

Dendritic Cell Subtype and Maturation

The maturation and subtypes of DCs are presumably a response to the encountered pathogen and the cytokine milieu, either in the peripheral lymph nodes via the lymph or in the spleen via the circulatory system. Nonactivated immature DCs are thought to continuously present self-antigens to autoreactive T cells in the absence of co-stimulation. This induces anergy or deletion of potentially harmful T cells (Fig. 2.8) [83]. If a microbe breaches the epithelium to enter connective tissue and parenchymal organs, it can be captured by an immature DC that reside in these tissues and be transported to the peripheral lymph nodes for antigen presentation to T lymphocytes. Recent studies indicate that soluble antigens directly diffuse into draining LNs via lymphatics and conduits, thereby reaching the resident DCs [84]. Despite their proficiency as APCs, during this process of migration into the lymph nodes, the activated DCs can undergo semi-maturation into tolerogenic DCs. Semi-mature DCs, in a steady-state, have demonstrated tolerogenic functions by skewing TH1/TH2 balance as well as generating and interacting with regulatory T lymphocytes (CD4+CD25+FOXP3), to suppress autoimmunity (Fig. 2.8) [85]. DCs become activated following the capture of antigens, triggering of toll-like receptors (TLRs) and the innate pro-inflammatory cytokine production. Activated DCs lose adhesiveness for epithelial tissue, but express surface

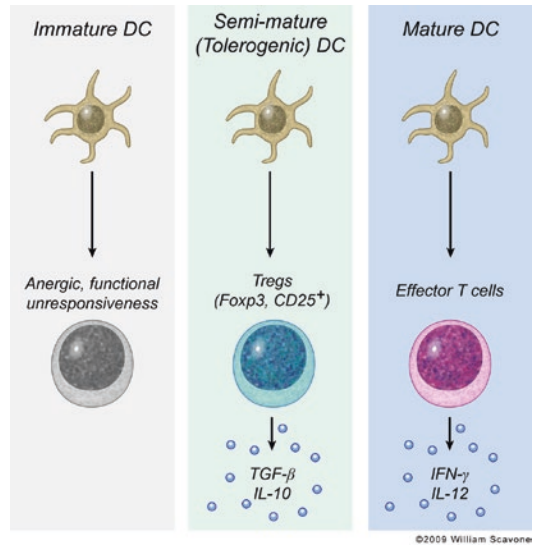

Fig. 2.8 DC maturation. DCs are referred to as immature prior to binding and endocytosing antigen, as they are inactive and inefficient at stimulating T lymphocytes. Semi-mature DCs have demonstrated tolerogenic functions by skewing TH1/TH2 balance (producing IL-10 and TGFβ) as well as generating and interacting with regulatory T lymphocytes (CD4+CD25+FOXP3), to suppress autoimmunity. Mature DCs are immunogenic once antigens are encountered, endocytosed, and presented to T lymphocytes in an inflammatory microenvironment, resulting in effector functions (production of IFN-γ and IL-2). (Reprinted with permission from William Scavone)

receptors for homing chemokines that direct the DCs into the lymph and peripheral lymph nodes. Antigen presentation of both MHC I and II, as well as expression of co-stimulatory molecules (CD80/CD86), and pro-inflammatory cytokines efficiently activate T lymphocyte effector functions and cytokine production of TNFα and IL-2 (Fig. 2.8) [86]. Traditionally, DCs have been referred to as mobile sentinels due to their capacity to capture antigen, migrate to LNs, and present to and activate lymphocytes. However, recent research has uncovered that DCs have the ability to minimize autoimmunity. Once these processes are better understood, they may be used to induce tolerance in autoimmune diseases.

Cytokines

Cytokines represent critical mediators of the autoimmune process. They are generally small molecular weight soluble proteins that are secreted and responsible for communication between leukocytes and between leukocytes and other cells. They bind to their cognate receptors to induce a signaling cascade [5]. Cytokines function in both an autocrine and paracrine manner to induce a number of cellular responses. There are currently 35 interleukins (ILs) that have been cloned and characterized, tumor necrosis factor (TNF), chemokines, interferon-γ (IFN-γ), and type 1 interferons α/β (T1IFNs). Many of the cytokines were found to be members of a family based on sequence similarity, sharing of subunits, sharing of receptors, or having cognate receptors that share subunits. For example, IL-2 is an important T cell cytokine produced at high levels by naive CD4 T cells following antigen recognition. It serves as a growth and survival factor for T cells. IL-2 binds to its receptor, called the IL-2 receptor (IL-2R), which can consist of up to three chains: α, β, and c. The combination of receptor components determines the affinity of IL-2 to its receptor. On naive T cells, the α-chain, also known as CD25, is rapidly upregulated following antigen recognition and in combination with the β and c chains forms a high-affinity receptor. The c chain, also called the IL-2R common c chain, is also a component of the IL-4, IL-7, and IL-15 receptors [5]. Monoclonal antibodies to cytokine receptors are being developed in order to suppress cytokine binding and proliferation of the cytokine production, leading to specific autoimmunity (i.e., daclizumab, anti-CD25 (IL-2R)). There are a number of cytokine families that influence T cell biology and could be targeted in autoimmune disorders.

Endogenous T1IFN is a naturally occurring regulatory cytokine that is ubiquitously expressed except on red blood cells. Interferon (IFN) is pivotal for bridging the innate and adaptive immune response, as it is produced in response to viral stimuli by innate cells (T1IFN and IFN-γ) as well as T lymphocytes (IFN-γ) [15]. The key cell type that produces T1IFN is plasmacytoid dendritic cells (pDCs) [82]. pDCs are induced by toll-like receptors (TLR) on APCs. The pleiotropic effects of IFN include potent antiviral activity, antiproliferation, and immunomodulatory activities on the immune system [87]. T1IFN can stimulate the transcription of many genes primarily through the Janus kinase (JAK)-STAT pathway. In addition to gene induction, T1IFN can also inhibit the transcription of selected genes, although less is known about the mechanisms underlying IFN-b-mediated negative gene regulation [88]. Cells targeted by T1IFNs include, but are not limited to, DCs, lymphocytes, macrophages, astrocytes, and neurons. Type I IFNs are differentially involved with a number of autoimmune disorders [87] and therefore intriguing therapeutic targets.

Stem Cells

Stem cells have varying potential as therapeutic targets for neurologic autoimmune disorders. While highly controversial, embryonic stem cells are considered truly pluripotent and most versatile for regenerative medicine; adult stem cells also hold therapeutic potential. They are multipotent and far less controversial. Stem cells have

common attributes that enable their self-renewal, survival, and maintenance of genomic integrity [89]. All tissues appear to have stem cells, and within each tissue type stem cells are located in a specialized vascular microenvironment called a "niche." Critical to the maintenance of the stem cell niche are microenvironmental cues and cell-cell interactions (cell adhesion molecules and integrins) that balance stem cell quiescence with proliferation, specification, and differentiation of progenitor cells [89, 90]. The microenvironment, a common theme in the homeostasis of immunity and health, plays a key role in the therapeutic potential of adult stem cells, whether endogenous or exogenous/transplanted.

Adult bone marrow contains at least three stem cell populations: hematopoietic stem cells (HSCs), mesenchymal stem/stromal cells (MSCs), and endothelial progenitor cells (EPCs) [89]. HSCs are rare among bone marrow cells, with a frequency of perhaps 1 in 10,000 or more [89]. Identification of HSCs is based upon the cell surface marker CD34+. Transdifferentiation/cell fusion [91, 92] is but one of many potentially therapeutic properties of adult stem cells and possibly one of the most important [93, 94]. Other potential mechanisms include but are not limited to dedifferentiation, transdetermination, true pluripotent stem cell behavior, and production of trophic factors [91]. The rationale behind autologous hematopoietic stem cell transplantation (HSCT) for MS, for example, is to induce new and self-tolerant lymphocytes (resetting the immune system) following chemotherapy-induced elimination of self-reactive lymphocytes [95]. Similar to malignancy response to HSCT, autoimmune diseases that respond to immunosuppressive therapy tend to respond to immunosuppressive conditioning followed by autologous HSCT rescue. Relapsing-remitting MS is an example of an inflammatory, immune responsive disease where an autologous HSCT study showed positive results in the form of 100% progression-free survival after a mean follow-up of 3 years (as defined by "no deterioration in their Expanded Disability Status Scale") [95]. In contrast, traditional immune nonresponsive diseases such as primary progressive MS and late secondary progressive MS show little to no improvement following autologous HSCT [96]. Current research is ongoing to review the risk-benefit of autologous HSCT as well as the optimal conditioning regime (complete/partial/non-myeloablation) prior to autologous HSCT [95–98].

MSCs have been studied in animal models and, following acute neurologic injury, migrate to the damaged brain [99]. MSCs can proliferate extensively in vitro and differentiate under appropriate conditions into bone, cartilage, and other mesenchymal tissues, as well as multiple other cells including neuroectodermal cells [99–101]. These results, albeit in animal models, suggest that human MSCs could provide an ideal cell source for repair of injured organs including the CNS. Studies with human MSCs have identified comprehensive immunomodulating properties [93]. Modulation of host immune responses due to low immunogenic properties [102, 103] and the ability to secrete neurotrophins provides a microenvironment that induces neuronal cell survival and regeneration. Transplantation of MSCs, similar to HSCT, provide the most benefit in acute neurological injury and/or early inflammatory stages of disease. MSCs are rare and decline with age, so that alternative sources of MSCs may be integral for allogeneic therapeutic application in the future, particularly MSCs isolated from human umbilical cord blood [104].

CNS stem cells have tri-lineage potential, capable of generating neurons, oligodendrocytes, and astrocytes. During CNS development the neuroepithelial cells in the embryonic ventricular layer generate most of the neurons and glia (astrocytes and oligodendrocytes). A consensus view is that astrocytes are the main stem cell population, with small numbers of neural stem cells (NSCs) in other regions [89]. The niche for these NSCs has been identified as the subventricular zone (SVZ) lining the lateral ventricles and the subgranular zone (SGZ) of the dentate gyrus of the hippocampus [105]. The neural stem cell niches define zones where stem

cells are retained after embryonic development for the production of new cells of the nervous system. This continual supply of new neurons and glia then provides the postnatal and adult brain with an added capacity for cellular plasticity, neurogenesis, and gliogenesis that is restricted to the SVZ and SGZ within the brain [90]. In EAE, prolonged inflammation extensively alters the proliferative and migration of endogenous NSCs in vivo [106]. In animal models research has demonstrated that transplanted NSCs migrate specifically to the injured CNS under the guidance of immune responsive cells, potentially directing targeted migration of stem cells toward the sites of inflammation/disease [107, 108]. Therapeutics aimed at facilitating endogenous or exogenous reparative processes will need to realize the timing of therapeutic potential, as it relates to stage and duration of disease process.

Remyelination represents one of the most compelling examples of adult multipotent progenitor cells contributing to the endogenous regeneration of the injured CNS [109]. This process has been noted to occur in the clinical disease MS [110], and the experimental disease EAE, revealing the impressive ability of the adult CNS to repair itself. The inconsistency of remyelination in MS, with loss of axonal integrity, makes enhancement of remyelination an important therapeutic objective. There is tremendous research in this area, looking to expand upon ways in which to improve specification, differentiation, translocation/mobilization, and function of endogenous NSCs and/or transplanted adult stem cells. The goal is to repair the degenerated or injured neuronal pathways [111, 112]. Autologous stem cell transplantation (HSC, MSC, NSC) may provide greater potential than just cell replacement. The concept of "therapeutic plasticity" refers to the capacity of stem cells to produce neuroprotection and immunomodulation in response to specific microenvironmental needs of different pathological conditions [108]. Pharmacological and cell-based restorative immunotherapies will need to demonstrate remodeling and enhancement of neurological function while providing an acceptable risk-benefit ratio.

The Gut Microbiome in Multiple Sclerosis

Among the environmental factors that have been associated with elevated risk for multiple sclerosis, the gut microbiome is perhaps the most significant. Although our understanding of the microbial world has been traditionally driven by the study of pathogenic microbes, most are non-pathogenic. We are surrounded and colonized by a complex community of microbes that include bacteria, archaea, viruses, fungi, and other microscopic eukaryotic cells. Collectively, the community of microbes found at a given system is named the microbiota, while the interactive combination of microbiota with the host is termed microbiome. Despite the direct and indirect negative impact of pathogenic microbes in health, we now understand that most microbes are symbionts with the host. While receiving nutrients and a physically defined space to survive, microbes reciprocally provide metabolic benefits to the host. New molecular, genetic, and animal experimental tools provide a novel framework for the study of the interactions between the microbiome and the host, and the complex multifactorial interactions are now beginning to be understood. The most studied microbiome is the gut microbiome of both mice and humans. Genetic, physical, and chemical factors shape the composition of the gut microbiome in healthy individuals, and as evidenced by the most recent findings, disease and disease-modifying therapies also affect such composition. In this section, we will highlight the most salient findings that suggest an interaction between the gut microbiome and animal models of multiple sclerosis and between the gut microbiome and the human disease.

The Anatomy of the Gut Epithelium in the Context of Disease

Because of the elevated amounts of microbes and microbial antigens present, the mucosa-associated to the gastrointestinal (GI) tract contains 80% of the host immune cells comprising the largest concentration of immune cells in the

body [113]. Despite its function absorbing nutrients and reabsorbing water sequentially, the gut epithelium establishes an effective barrier against microbes serving as a physical and chemical separation between the lumen of the gut and the internal tissues, by the effects of the mucus produced by goblet cells and antimicrobial peptides produced by Paneth cells residing within the crypts of the small and large intestine (although much reduced in numbers in the large intestine).

As mentioned before because of the elevated numbers of microbes and derived antigens and metabolites, the immune system is explicitly present in the gut, forming the gut-associated lymphoid tissues (GALT). The proposed function for the GALT is the constant surveillance for pathogens and the maintenance of immune tolerance to beneficial members of the microbiome. As a combined secondary lymphoid tissues associated with the gut mucosa, the GALT is composed of diffuse lymphoid follicles, more organized Peyer's patches, and lymphatics that drain into structurally defined mesenteric lymph nodes (MLN) through afferent lymphatic vessels. Antigen-presenting cells that sample the gut as well as soluble antigens traffic through the MLN on a CCL21- and CCR7-dependent mechanism where they encounter naïve T cells that entered the lymph nodes through the high endothelial venules from circulation. Within the lymph nodes T cell activation, clonal expansion, and differentiation occur. Differentiation into specialized effectors is necessary for generating specific responses against microbes. Peripheral tolerance also occurs within the GALT, by the direct effects of regulatory T cells (Tregs) or by anergy.

The GALT as a Regulator of Immune Function

T helper (Th) cells such as Th1, Th2, and Th17 help mobilizing and recruit innate immune cells against microbes. However, in the gut, there is a very pressing need of activating active and passive mechanisms of peripheral tolerance in order to control inflammatory responses against nonpathogenic and potentially beneficial microbes. Immune cells will render nonresponsive in the absence of appropriate co-stimulation or be controlled by Tregs. Some gut symbionts, such as *Bacteroides fragilis*, promote immunotolerance by directing the production of interleukin-10 (IL-10)-producing Tregs in the GALT through the recognition of dendritic cells of polysaccharide A (PSA) [114] described later in further detail. Anergy is another mechanism of peripheral tolerance characterized by the lack of an immunological response to antigen. Although the mechanism remains to be elucidated, gut symbionts could promote incomplete activation of T cells with no co-stimulatory signal that would result in nonresponsive T cells [115].

Due to the importance of the gut microbiome shaping immune responses, many labs across the world have focused their efforts on understanding the association between the microbiome and diseases, with particular emphasis on autoimmune diseases hypothesized to be linked to immune functional deficiencies. Furthermore, the gut microbiome composition is affected by factors including host genetics, geographical location, diet, lifestyle choices, prescribed pharmaceuticals, mode of delivery during birth, antibiotic exposure, and others that have been previously proposed to impact the risk for diseases. Specifically, the concept of dysbiosis proposes that factors that unbalance the composition of the gut microbiome result in changes in immune function that can lead to disease. The paradigm that an unbalanced gut microbiome could shift a homeostatic immune system toward a pro-inflammatory state could exacerbate functional dysfunction associated with the peripheral tolerance in MS patients. Furthermore, the disruption of the integrity of the intestinal barrier promotes endotoxin and bacterial translocation that exacerbates systemic inflammation [116], which in turn could impact significantly CNS immunity and the integrity of the blood-brain barrier [117].

In MS, Tregs are defective in their ability to control the proliferation of pro-inflammatory, autoreactive, T cells [118, 119]. Since demyelination could be potentiated by the effects of Th17 cells by the secretion of pro-inflammatory media-

tors that recruit pathogenic cells, and both Treg and Th17 cell balances are impacted by the composition of the gut microbiome, recent efforts have put special emphasis on dysbiosis in the context of MS.

The Gut Microbiome of Multiple Sclerosis Patients

The first studies designed to evaluate the potential association between the gut microbiome and CNS inflammatory demyelination that characterizes MS used the murine experimental autoimmune encephalomyelitis (EAE); most used animal model to study MS. First, we reported a number of years ago that the oral administration of a mixture of broad-spectrum antibiotics reduced the severity of EAE in mice, by reshaping the balance between pro-inflammatory and Tregs [120] and by impacting the function of gut-derived natural killer T (NKT) cells [121]. Antibiotics have also been shown to impact the severity of other models of autoimmunity such as experimental autoimmune uveitis [122]. In this later study, the treatment with antibiotics significantly increased the expression of Tregs and reduced IL-17-producing Th17 cells [122], similar to the studies performed in EAE mice [120].

The impact of the gut microbiome regulating the severity of EAE was later confirmed in studies using germ-free (GF) mice, wherein mice that are born and raised under strict sterile conditions are unable to mount an inflammatory CNS demyelinating condition. It was previously shown that GF mice show reduced frequencies of gut-derived Th17 cells [123] that impacts their susceptibility to a variety of experimental autoimmune diseases such as diabetes [124], inflammatory bowel disease (IBD) [125], RA [126], and EAE [127, 128] when compared with conventional housed animals. In EAE and some other experimental conditions, GF mice exposed to the monocolonization with segmented filamentous bacterium (SFB), a known inducer of Th17 cells in the GALT, restored susceptibility to disease, consistent with what has been observed in conventionally housed mice [126, 128].

In MS patients, significant modifications of specific microbial taxa have been observed [129–131]. Although overall the broad composition of the microbiome remains unaffected in MS patients when compared to healthy individuals, a more profound view of a multitude of microbial taxonomical units shows patterns associated with specific changes that could lead to dysbiosis. Moreover, recent evidence suggests a functional impact of the gut microbiome those with MS. Two recent works demonstrate that the fecal transplantation of dysbiotic MS gut microbiome into GF mice restores the susceptibility of these mice to EAE [132, 133].

Causality as to whether the microbiome changes are responsible for the disease state or conversely the consequence of disease remains to be elucidated [134]. Effects of immunomodulatory therapeutics that target immune cells associated with the immunopathology of MS on the gut microbiome have been demonstrated. Furthermore, EAE studies in nonobese diabetic mice have shown that active induction of disease promotes significant changes on the microbiome that are most apparent at early stages of the disease [135]. Remarkably, the early treatment of EAE mice with antibiotics reduced the severity of the disease while later treatments did not affect the progression of disease.

The increase in the understanding of the interactions between the microbiome and disease offers alternative venues for the development of newer therapeutics. While microbes of the oral microbiome such as *Porphyromonas gingivalis* exacerbates EAE [136, 137], other components of the microbiome or even microbial products show promising immunomodulatory effects that result in reduced severity in experimental models of disease. *Bifidobacterium animalis* reduces rat-induced EAE [138] and *Lactobacillus* spp. are protective against EAE in mice in a mechanism dependent on the induction of IL-10-producing Tregs [139]. A similar mechanism of action has been proposed for the protective effects of *Prevotella histicola*, a common member of the human gut microbiome that reduces Th1 and Th17 cell function by promoting tolerance [140]. Another member of the human gut microbiome,

Bacteroides fragilis, expresses eight capsular polysaccharides, one of which, the zwitterion polysaccharide A (PSA), promotes protection against different autoimmune experimental models by IL-10-producing CD4+ T cells (both Foxp3 positive and negative) [141]. PSA is considered a symbiotic factor since its production facilitates the survival of the microbe in the gut and also promotes the induction of population regulatory CD4+ T cells, including Tregs [141, 142], Foxp3-negative CD4+ T cells [143], and CD39+ T cells that may or may not express Foxp3 [144, 145] that suppress the pro-inflammatory cell populations. In the context of EAE PSA is protective [142, 144, 145]. Furthermore, PSA is capable of promoting a regulatory phenotype in human peripheral blood mononuclear cells (PBMCs) isolated from both healthy individuals [146] and MS patients [147]. The regulatory function of the human regulatory T cells promoted by PSA is associated with an enhanced IL-10 production that has been shown to suppress TNF-α production by monocytes stimulated with LPS in vitro [146]. The later studies demonstrate that gut microbiome-derived symbiont factors promote immunomodulatory responses in EAE mice and in samples isolated from MS patients, opening new possibilities in the search for novel therapeutics. Thus, the gut microbiome represents a unique and truly novel "treasure trove" of potential metabolites and antigens that may serve as a profound basis for future therapeutic intervention in a wide range of human disease including autoimmunity and multiple sclerosis.

Conclusion

Bench and clinical research focused on autoimmunity have provided abundant details related to the pathogenesis of many neurological diseases and a greater understanding of the current and novel treatment approaches to regulate the immune system. Much remains in question. The immune microenvironment drives cellular response. In order to re-establish immune homeostasis and regain tolerance, it will require the concerted action of multiple cell types. If any one of the cell types is missing, peripheral tolerance will be avoided. The possibility of a single therapeutic agent, directed at a single target, resolving the complex interactions in disease pathogenesis may not be attainable. It may take multiple targets, treated simultaneously or serially, in order to restore the homeostatic balance needed for disease resolution. Restoration of the dysfunctional immune response will in all likelihood require careful dissection and manipulation rather than a sweeping ablative therapy that could be harmful. More bench and clinical research is needed to study other therapeutic targets such as allogeneic HSCT, antimetabolites, toll-like receptors, statins, vitamins D and A (retinoic acid), commensal bacteria, continued genomic evaluation, and individualized treatments regimes.

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