

Dysregulation of immune homeostasis in autoimmune diseases

Vijay K Kuchroo, Pamela S Ohashi, R Balfour Sartor & Carola G Vinuesa

Great strides have been made in our understanding of the pathogenesis of autoimmune diseases. Research has identified genetic associations, new functional subsets of effector and regulatory T cells and the gut microbiota as crucial environmental factors in regulating immune function. This commentary discusses recent advances in our understanding of genetic and environmental factors as well as the role of innate and adaptive immune responses in driving immune-mediated tissue injury.

Gene-environment interactions

Genetics of autoimmune and immune-mediated diseases. The occurrence of multiple affected individuals in families, the increase in relative risk of disease in non-identical twins and the comparatively higher incidence of disease in identical twins suggest that there is a genetic component to immune-mediated diseases. With the availability of single-nucleotide polymorphism

(SNP) and haplotype maps of both the human and mouse genomes together with genome-wide association scans, there has been an explosion in the last decade in the number of genes found to be associated with human autoimmune diseases. From these genetic analyses, the major histocompatibility complex (MHC) region on chromosome 6 stands out as the most crucial susceptibility locus and is associated with the highest number of human autoimmune diseases¹. In addition to the MHC, over 100 different non-MHC genes that contribute to the susceptibility of developing an immune-mediated disease have been identified, but the overall contribution of these genes to their respective diseases is minor. Nevertheless, the non-MHC genes that have been identified are mostly genes of the immune system, confirming that these disorders are indeed a product of allelic variation in immune-related genes and not of degenerative diseases². Recently identified examples of non-MHC genes include polymorphisms in the interleukin-23 receptor (IL-23R) in multiple diseases and in genes associated with innate immune function, notably nucleotide-binding oligomerization domain containing 2 (NOD2) in Crohn's disease (**Fig. 1**)³. The prevailing hypothesis, for which there is some evidence, suggests that these allelic variations in immune genes did not evolve to increase predisposition to autoimmune diseases but, rather, were selected for as a result of environmental pressures (for example, infections) because they conferred a selective survival advantage in a highly infectious environment⁴.

Because the MHC is one of the most dominant genetic elements that predisposes to autoimmunity, this raises the issue of how the MHC locus predisposes an individual to developing autoimmune disease. Some MHC molecules may not be able to efficiently mediate the deletion of T cells that recognize

self-antigen and are therefore more permissive to the development of self-reactive T cells in the thymus⁵. The molecular basis of how certain MHC molecules are unable to effectively drive the deletion of self-reactive T cells is now beginning to be identified through studies of the crystal structures of self-reactive T cell receptor (TcR)-peptide-MHC complexes. There are alterations in the structure of these complexes that may result in either suboptimal interactions or suboptimal recognition of the self-peptide-MHC by the autoreactive TcRs⁶. These suboptimal TcR-peptide-MHC interactions allow self-reactive T cells to escape deletion and seed the peripheral immune compartment where the self-reactive T cells can be activated by infections or other environmental agents.

Environmental factors. Despite the contribution of genetic factors, immune-mediated diseases rarely reach above a 40% incidence in monozygotic twins⁷, suggesting that non-genetic or environmental factors also have a role in the development of immune-mediated diseases. Environmental factors (for example, infections, vitamin D, smoking or microbiota^{8,9}), epigenetic mechanisms and somatic mutations all contribute to disease discordance between monozygotic twins, but these factors are for the most part not well characterized. Identification of environmental factors that predispose to autoimmune diseases has been difficult, but thorough epidemiological studies together with long-term follow-up studies are beginning to identify the environmental triggers that induce autoimmune disease. These studies, together with genetic linkage studies of the same populations, are providing additional insight into the mechanism by which an environmental trigger may precipitate an autoimmune disease on a defined genetic background (**Fig. 1**). A case in point is the finding that exposure to cigarette smoke on an appropriate MHC background increases

Vijay K. Kuchroo is at the Center for Neurologic Diseases, Brigham and Women's Hospital, Harvard Medical School, Boston, Massachusetts, USA. Pamela S. Ohashi is at the Campbell Family Institute for Breast Cancer Research, Ontario Cancer Institute, Departments of Medical Biophysics and Immunology, University of Toronto, Toronto, Ontario, Canada. R. Balfour Sartor is at the Departments of Medicine, Microbiology and Immunology, Center for Gastrointestinal Biology and Disease, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina, USA. Carola G. Vinuesa is at the John Curtin School of Medical Research, Australian National University, Canberra, Australian Capital Territory, Australia.
e-mail: vkuchroo@rics.bwh.harvard.edu

the risk of rheumatoid arthritis by 20-fold⁸. In the subgroup of individuals with rheumatoid arthritis who smoke, smoking is believed to citrullinate a subset of proteins (including enolase and vimentin, among others) in the lung, which induces an arthritogenic immune response in individuals that have a defined set of MHC class II molecules (human leukocyte antigen (HLA)-DRB1 molecules that present the shared epitope)⁸. The presence of serum antibodies to citrullinated proteins (termed anti-citrullinated protein antibodies) has provided a crucial biomarker for the diagnosis of rheumatoid arthritis in this subset of individuals and can be used to stratify patients and predict the outcome of certain interventions. The interplay between genetic susceptibility and an environmental trigger is further illustrated by interactions between a risk allele for Crohn's disease, ATG 16L1 and noravirus, a common enteric viral pathogen. In a study by Cadwell *et al.*¹⁰, hypomorph mice with the ATG 16L1 susceptibility allele that were infected with a particular strain of murine noravirus showed increased susceptibility to induced colitis, which was in contrast to the low susceptibility to colitis seen in noninfected ATG 16L1 hypomorph mice, ATG 16L1 hypomorph mice infected with a different noravirus strain and wild-type mice infected with the colitogenic noravirus. These results suggest a specificity of both the host genetic factors and the infectious triggers contributing to disease susceptibility and emphasize that on a defined genetic background, a specific environmental trigger becomes a risk factor for developing autoimmune disease.

As with smoking and infections, environmental toxins are known to trigger autoimmune diseases. A well-known environmental toxin, dioxin, has been shown to modulate immune responses, but the molecular basis for this effect is just beginning to be defined. Dioxin binds to the aryl hydrocarbon receptor (AHR), a ligand-dependent transcription factor that is differentially expressed in different subsets of T cells¹¹. In addition, AHR has been shown to be expressed in dendritic cells as well¹². AHR is expressed in both highly proinflammatory type 17 T helper (T_H17) cells and regulatory T cells (both forkhead box P3-positive (Foxp3⁺) regulatory T (T_{reg}) and type 1 regulatory T (Tr1) cells)^{11,13–15}. The nature of the AHR-driven immune response depends on the specific AHR ligand. Some ligands (such as 6-formylino[3,2-b]carbazole) activate proinflammatory T_H17 cells and induce autoimmunity, whereas others (such as kynurenine or TCDD) activate T_{reg} and Tr1 cells and suppress autoimmunity^{11,13,16,17}. Although it is unclear how

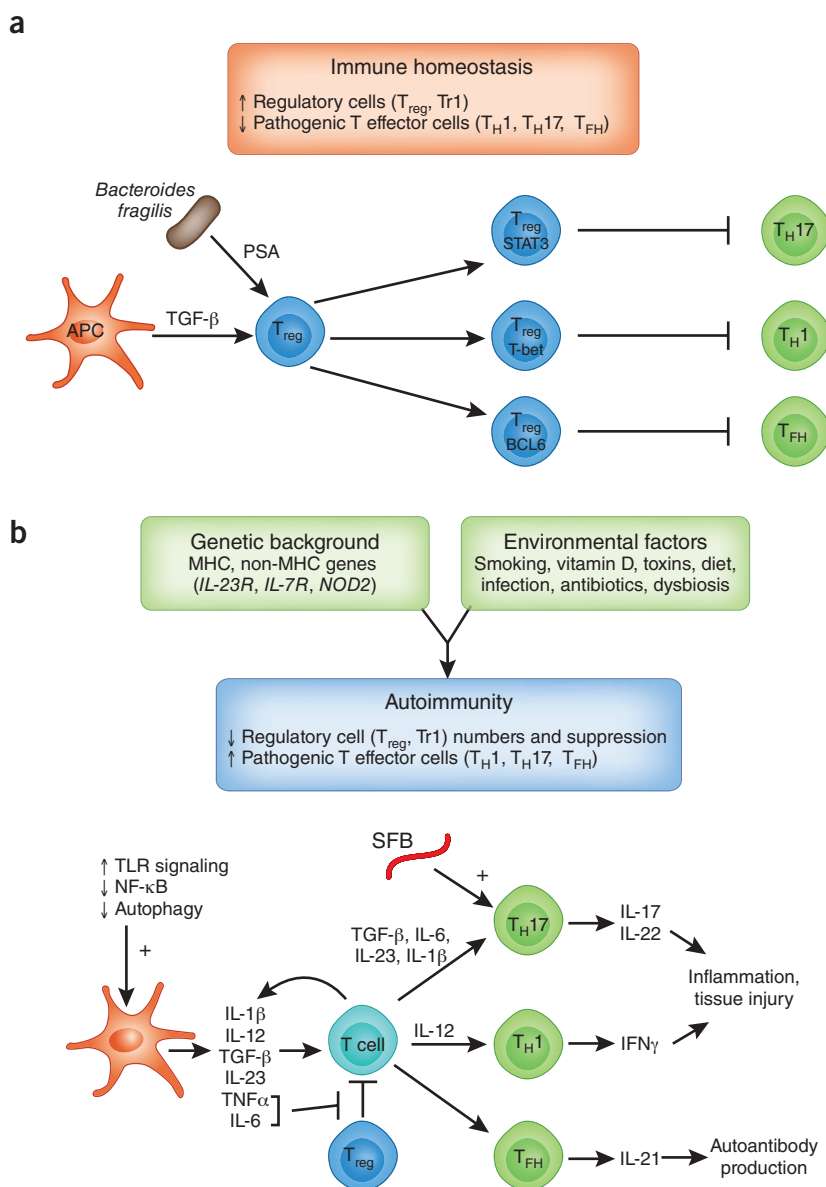


Figure 1 The balance between regulatory and pathogenic effector T cell subsets in health and autoimmune disease. **(a)** In conditions of immune homeostasis, APC-derived TGF- β promotes the development of Foxp3⁺ T_{reg} cells, which then further specialize. By expressing the transcription factors, T-bet, STAT3 and BCL6, T_{reg} cells can specifically suppress effector T_H1 , T_H17 and T_{FH} cell responses, respectively. The development of T_{reg} cells can also be directly promoted by PSA derived from *B. fragilis*. **(b)** A combination of host genetic factors and exposure to environmental triggers promotes the development of autoimmune disease. APCs can be activated by numerous factors, resulting in the release of cytokines that promote the differentiation of naive T cells into pathogenic effector T cell subsets, which drive inflammation, tissue injury and autoantibody production. Segmented filamentous bacteria (SFB) can also promote the development of T_H17 cells and autoimmune responses *in vivo*. Pro-inflammatory cytokines derived from resident innate and adaptive immune cells, including TNF- α and IL-6, attenuate T_{reg} cell-mediated suppression of effector T cells.

these AHR ligands affect the innate immune responses, these data provide support for a mechanism in which environmental toxins, by binding to specific receptors expressed in the immune system, induce or suppress effector immune responses.

Perhaps the environmental factor with the most substantial effect on immune function

and the development of immune-mediated diseases that has been identified in the last few years is the commensal gut microbiota. The gut harbors trillions of microbes, which are in close contact with the gut mucosa¹⁸. The gut microbiota develop a symbiotic relationship with the immune system; however, their presence is not entirely innocuous but,

rather, has a major impact on the development of immune responses even at distal sites. The composition of gut microbiota, and even the presence or absence of a single microbial species in the gut, can regulate the balance between effector and regulatory T cells in a host- and strain-dependent manner. For example, a single species of anaerobic bacteria, segmented filamentous bacterium (SFB), which is present in high numbers in some animal colonies, promotes the generation of T_H17 cells in the gut (Fig. 1b)¹⁹. Monocolonization with SFB was sufficient to induce T_H17 responses in mice that do not normally have a high frequency of T_H17 cells¹⁹ and can precipitate the development of arthritis in the F1 crosses of KRN T cell receptor (TCR) transgenic mice (K/B × N mice)²⁰. Similarly, other microbial species in the microbiota, independent of SFB, were shown to trigger autoimmune demyelinating disease in mice expressing myelin-reactive T and B cells²¹. Non-obese diabetic (NOD) mice develop an autoimmune response against β islet cells and develop type 1 diabetes spontaneously. However, natural SFB infection in diabetes-susceptible NOD mice was shown to segregate with protection against diabetes²². From these data, it is clear that the same bacteria can either promote or inhibit autoimmunity, depending on the type of disease and, perhaps, the different host genetic characteristics. This might reflect the requirement for different subsets of effector T_H cells. Therefore, these data raise caution that simple replenishment or colonization with a defined bacterial species may suppress one autoimmune disease but trigger another on a genetically susceptible background. Furthermore, these studies show that variable colonization by gut-resident commensals within a single mouse colony can explain the incomplete penetrance of an autoimmune disease. Consistent with the idea that the gut might be a compartment with unique properties, which can either trigger or dampen the differentiation and activation of pathogenic T_H17 cells, it was recently shown that T_H17 cells can be controlled in the gut. T_H17 cells can be eliminated by the intestinal lumen or can attain a regulatory phenotype and produce IL-10 (ref. 23). Whether induction of IL-10 in T_H17 cells in the gut is also dependent on the presence of unique commensals in the gut of these mice has not been investigated.

In addition to the induction of proinflammatory effector T cells, other bacterial species have been shown to induce T_{reg} cells that have the potential to suppress immune-regulated diseases. Polysaccharide A (PSA)

from *Bacteroides fragilis* was shown to induce IL-10 and Foxp3⁺ T_{reg} cells through a Toll-like receptor 2 (TLR2)-dependent mechanism (Fig. 1a)^{24,25}. PSA acts directly on Foxp3⁺ T_{reg} cells and expands them to promote immunologic tolerance²⁵. In contrast, *B. fragilis* lacking PSA were unable to expand Foxp3⁺ T_{reg} cells and instead promoted the expansion of proinflammatory T_H17 cells. In addition, lipoteichoic acid from *Lactobacillus acidophilus* can suppress the production of IL-12 and tumor necrosis factor α (TNF- α) from innate immune cells²⁶.

A number of factors, including early familial exposure, mode of delivery (vaginal birth or caesarean section), diet, genetic background and antibiotics, contribute to shaping the composition and function of the microbiota²⁷. Not surprisingly, diet has been shown to dynamically alter the composition of gut microbiota, with a high-fiber diet promoting the growth of protective *Bacteroides* species in the gut²⁸. In a recent study, long-term dietary patterns were shown to determine broad microbiota profiles, or enterotypes, with a high fat and low fiber diet correlating with a *Bacteroides*-dominated enterotype and a low fat and high fiber diet promoting a *Prevotella* enterotype²⁹. Short-term alterations affected the composition of the microbiota but not the enterotype, showing that long-term dietary modification may be necessary for sustained effects in the gut. Several additional factors, such as body mass index and the consumption of red wine, affected the microbial composition, which may help explain the increased prevalence of immune-mediated diseases in developed countries^{29–31}. The loss of key immune genes (for example, the genes encoding T-box 21 (T-bet), NOD-like receptor family pyrin domain containing 6 (NLRP6) inflammasome and NOD 2) has been shown to induce dysbiosis and promote the development of colitis, intestinal hyperplasia and colonic tumors, again suggesting that an intimate relationship exists between the microbiota and the immune system^{32–34}.

Although the intestinal microbiota can shape the immune system, the immune system also changes the composition of microbial species that reside in the gut²⁷. This relationship is further illustrated by the higher incidence of ulcerative colitis and Crohn's disease in Indian children born and raised in geographical environments outside of India (Britain, Canada and the United States), where exposure to infectious agents may not be as high may be different than in their native India^{35,36}, as compared to children born and raised in India. It has been suggested that exposure to a high infec-

tious load early in childhood in India selects 'vigilant genotypes', in which individuals with a combination of different allelic variants mount a potent immune response to gut-associated pathogens, preventing early mortality as a result of gastrointestinal infections³⁷. The immune system may be 'tuned' or modified by early childhood infections that lead to the generation of T_{reg} cells in the gut. Later in life, these T_{reg} cells may ensure that the immune system does not mount too vigorous a response toward the gut microbiota. However, the genetic makeup of an individual may be maladapted in a different geographical environment than the one into which the individual was born, which may result in microbial dysbiosis leading to inflammatory bowel disease (IBD)³⁷. This hypothesis suggests that individuals with a different genetic makeup than the general population where they reside who are brought up in an environment where infectious exposure early in childhood is not very high or is different than in their native environment may be predisposed to developing IBD. The dominant influence of intestinal bacteria in IBD is strongly supported by the lack of colitis and bacterial antigen-specific T_H1 and T_H17 responses in multiple genetically susceptible mouse and rat strains raised in sterile (germ-free) environments but that showed rapid immune activation and onset of colitis after colonization with specific pathogen-free (commensal) bacteria³⁸.

Dysregulation of innate immunity

The innate immune system has a crucial role in driving the adaptive arm of the immune system to either promote or inhibit autoimmune disease. The resting innate immune system maintains self-tolerance, but once activated, innate immune cells can upregulate co-stimulatory molecules and produce cytokines that trigger the effector arm of the adaptive immune system (Fig. 1), generally to clear infections³⁹. However, a dysregulated innate immune response to pathogens, toxins or commensal microbiota can initiate a chronic inflammatory response. The activated innate immune system has to present autoantigens or microbial antigens in conjunction with appropriate co-stimulatory molecules and cytokines to overcome natural regulatory mechanisms and to trigger inflammatory responses driven by T and B cells. Experimentally, this mechanism has been shown in several transgenic models^{40–43}. In one model, the expression of viral glycoprotein in β islets did not trigger autoimmunity even in the presence of a high frequency of transgenic T cells expressing specific receptors for glycoprotein unless mice

were given the specific glycoprotein peptides together with stimuli that activated antigen-presenting cells⁴¹.

Both cell-intrinsic and receptor-driven mechanisms act to limit the activation of innate immune cells and maintain self-tolerance^{44,45}. Defects in some of the pathways that regulate the innate immune response (such as nuclear factor- κ B1 (NF- κ B1)) can overcome the need for dendritic cell maturation, thus promoting the development of autoimmunity⁴⁶. Similarly, alterations in other negative regulatory circuits (such as microRNAs or phosphatases that regulate TLR signals), which normally maintain dendritic cell activation and homeostasis, can bypass the natural 'brakes' and activate the innate immune system and promote autoimmunity^{47–49}.

Disruptions in the function of innate immune cells triggered by cell-extrinsic signals can also interfere with immune tolerance. For example, polymorphisms in the TNF-receptor superfamily have been associated with human immunoregulatory diseases, including IBD, systemic lupus erythematosus (SLE) and rheumatoid arthritis. There were significant differences in the carrier frequency for haplotype AT (adenine at 1466 and thymine at 1493) in the *TNFRSF1B* gene, encoding TNF receptor 2, between affected individuals⁵⁰. Therefore, dysregulation of innate pathways may overcome the controls necessary for maintaining tolerance and induce autoimmunity.

Allelic variations of innate immune receptors (TLRs, NOD-like receptors (NLRs) and NOD2), or an increase in their copy number, are associated with human immune-mediated and autoimmune diseases⁵¹. Genetic variation in the loci that affect the innate immune responses alter the duration, magnitude and quality of the effector T and B cell responses that are generated. The best example of this is the association of polymorphisms in TLR7 (which binds single-stranded RNA) with increased susceptibility to SLE^{52,53}. In mice, increased expression of TLR7 caused by gene duplication was also shown to accelerate the progression of systemic lupus-like disease⁵⁴. This is consistent with experimental results supporting the idea that B cells specific for RNA or DNA can bind to and internalize the autoantigen. This process activates and expands B cells, resulting in the production of more autoantibodies and the subsequent crosslinking of TLR7 and TLR9, a phenomenon called 'loop pathogenesis'⁵⁵. In such a situation, an autoantigen can also become an adjuvant to promote autoimmunity⁵¹. Similarly, one of the strongest genetic influ-

ences in Crohn's disease is *NOD2*, which encodes an intracellular receptor for the ubiquitous bacterial peptidoglycan component muramyl dipeptide. This observation and the association of multiple SNPs in autophagy genes with immune-mediated disease, such as *ATG16L1* and *IRGM*, have led to the hypothesis that lack of clearance of intracellular bacteria leads to the compensatory induction of the aggressive T_H1 and T_H17 responses to bacterial antigens that result in chronic intestinal inflammation^{3,38}. Individuals with Crohn's disease who have a *NOD2* frame shift mutation did not recruit *ATG16L1* to the plasma membrane at the site of bacterial entry⁵⁶. Individuals expressing an *ATG16L1* risk allele associated with Crohn's disease are defective in autophagy, bacterial trafficking and antigen presentation⁵⁷. These findings suggest a fundamental role for NOD proteins in bacterial sensing and the induction of autophagy, therefore linking *NOD2* to *ATG16L1*, two of the genes genetically associated with Crohn's disease.

Dysregulation of adaptive immunity

Dysregulation of the adaptive immune system lies at the core of autoimmune and immune-mediated disease pathogenesis. Hyperactivation of self-antigen- or microbial-antigen-specific effector T and B cells, paired with defects in the regulatory arm of the adaptive immune system, result in the breakdown of immune homeostasis and the development of immune-mediated diseases. As a result, the mechanisms by which effector and regulatory adaptive immune cells are dysregulated is a topic of intense research.

To induce an autoimmune response, effector T cells first have to acquire a defined cytokine phenotype after activation in the lymph nodes and then need to traffic to appropriate target organs where they induce tissue inflammation (Fig. 1). Interferon- γ -producing T_H1 cells have long been associated with organ-specific autoimmune diseases⁵⁸, however, recent studies have defined the importance of IL-17-producing T_H17 cells in driving autoimmune responses⁵⁹. Recently, a new subset of T_H cells called follicular T_H (T_{FH}) cells has also been identified. These cells provide help to B cells, induce germinal center reactions and promote the formation of long-lived antibody responses⁶⁰. T_{FH} cells are defined by both a specific cell-surface receptor profile (CD4⁺ inducible T cell costimulator (ICOS)^{hi} C-X-C chemokine receptor type 5 (CXCR5)^{hi} Programmed death-1 (PD-1)^{hi}) and by the expression of the lineage-specific transcription factor B-cell lymphoma 6 (Bcl6)⁶¹. Spontaneous overactivation or expansion

of T_{FH} cells causes autoantibody production and the development of lupus-like disease in experimental animals⁶². In fact, a subset of individuals with SLE have been shown to express high concentrations of circulating T cells that resemble T_{FH} cells, and this increased frequency of T_{FH} cells correlated with both disease severity and end-organ damage⁶³. Tight control of the frequency and function of T_{FH} cells is therefore necessary to suppress the development of autoimmune lupus-like disease.

In conditions of immune homeostasis, the actions of autoreactive T_H1 , T_H2 , T_H17 and T_{FH} cells are countered by Foxp3⁺ natural regulatory T (n T_{reg}) cells, inducible regulatory T (iT_{reg}) cells and even regulatory B cells that produce suppressive cytokines such as IL-10 and transforming growth factor β (TGF- β)⁶⁴. n T_{reg} cells are crucial in suppressing effector T cells in the draining lymph nodes and in inhibiting tissue inflammation in the target organ⁶⁴. Although all n T_{reg} cells express the transcription factor Foxp3, it has become clear that these Foxp3⁺ T_{reg} cells further specialize, express defined transcription factors and suppress distinct subsets of effector T cells. Deletion of the transcription factors T-bet, interferon regulatory factor 4 (IRF4) or signal transducer and activator of transcription 3 (STAT3) in Foxp3⁺ T_{reg} cells results in the generation of T_{reg} cells that are unable to suppress the T_H1 , T_H2 and T_H17 responses, respectively (Fig. 1)⁶⁵. In addition, an n T_{reg} cell population with the specialized function of suppressing T_{FH} cells has been identified, and cells in this population have been named T follicular regulatory (T_{FR}) cells^{66–68}. In addition to expressing Foxp3, T_{FR} cells express the transcription factor Bcl6, are suppressive *in vitro* and inhibit T_{FH} cells and germinal center formation *in vivo*. These observations support the hypothesis that functionally specialized subsets of Foxp3⁺ T_{reg} cells act to suppress defined subsets of effector T cells. Further, these findings suggest that it may be possible to develop T_{reg} cell-based therapeutic modalities that can suppress immune responses with a high degree of specificity in a subset-specific manner rather than by inducing generalized immune suppression.

As described above, Foxp3⁺ T_{reg} cells specialize to regulate specific types of effector lymphocyte responses. As T_{reg} cells induce tolerance and inhibit tissue inflammation, it is perhaps not surprising that they also specialize in the tissue niches. A recently identified example of tissue-specific T_{reg} cells is adipose-tissue-resident Foxp3⁺ T_{reg} cells⁶⁹. These T_{reg} cells found in fat acquire a distinct

molecular signature after trafficking to the adipose tissue in addition to expressing most of the canonical nT_{reg} cell signature genes that are generally observed in lymph-node-derived nT_{reg} cells. The amount of T_{reg} cells in fat is lower in obesity in both humans and mice and affects metabolic parameters, including insulin resistance, as a result of diet-induced obesity⁷⁰. Another example of tissue-specific T_{reg} cells are mucosal IL-10-secreting Tr1 cells that do not express Foxp3 but are activated by intestinal bacteria and suppress T_H1- and T_H17-activated intestinal inflammation⁷¹.

These data raise an interesting question: can peripheral lymphoid-derived Foxp3⁺ nT_{reg} cells further differentiate at the sites of tissue inflammation in autoimmune disease? Emerging data suggest that this is indeed the case. Comparing a whole-genome microarray analysis of Foxp3⁺GFP⁺ T_{reg} cells obtained from the site of inflammation with a whole-genome analysis of lymph-node-derived Foxp3⁺GFP⁺ T_{reg} cells, the Foxp3⁺ T_{reg} cells isolated from the inflamed central nervous system had acquired many of the characteristics and secreted cytokines produced by effector T cells in the central nervous system (T. Korn & V.J.K., unpublished data). Whether these T_{reg} cells are functionally impaired in terms of their ability to suppress autoreactive T cells and tissue inflammation has not been completely resolved. Interestingly, the T_{FR} cell-suppressive T_{FR} cells described above have also been shown to share characteristics with both nT_{reg} cells and T_{FR} cells^{66,67}.

A decrease in the frequency, function or both of Foxp3⁺ T_{reg} cells is often seen in autoimmune disease. This raises the issue of whether autoimmune diseases can be suppressed simply by passive transfer and an increase in the frequency of regulatory T cells. Despite the fact that Foxp3⁺ T_{reg} cells are enriched in the inflamed joints of individuals with rheumatoid arthritis, autoimmune-mediated joint destruction seems to progress unabated in these individuals⁷². This may be a result of the fact that the effector T cells also diversify and acquire additional functional characteristics at the site of tissue inflammation that are not observed in effector T cells in the draining lymph nodes⁷³. A functional analysis of T_{reg} cells obtained from inflamed target tissue showed that these T_{reg} cells are not able to effectively suppress the effector T cells that are present at the site of tissue inflammation even though they can suppress effector T cells obtained from other compartments (the lymph nodes, spleen or blood) of affected individuals⁷³. IL-6 and TNF- α at the site of tissue inflammation⁷³ can also pro-

tect effector T cells from T_{reg} cell-mediated suppression, perhaps by blocking TGF- β signaling (Fig. 1b)⁷⁴. Indeed, neutralization of both IL-6 and TNF- α renders inflamed, tissue-derived effector T cells susceptible to Foxp3⁺ T_{reg} cell-mediated suppression⁷³. This is consistent with the observation that neutralization of TNF- α in individuals with rheumatoid arthritis results in the recovery of CD4⁺CD25⁺ T_{reg} cell function⁷⁵. T_{reg} cells may not be able to exert their suppressive function effectively in the face of inflammation and, therefore, local inflammation in the target tissue has to be controlled for T_{reg} cells to mediate long-term resolution of immune-mediated diseases. Thus, in order to induce long-lasting remission of immune-mediated diseases, two factors have to be taken into account: control of inflammation and boosting the frequency and function of T_{reg} cells.

Looking forward

Although many immune-based therapies that suppress tissue inflammation (for example, treatment with antibodies to TNF- α or with cytotoxic T lymphocyte antigen 4 immunoglobulin (CTLA4-Ig)) have become available in the last decade, none is able to induce complete and sustained remission of autoimmune diseases. Complete remission should be our long-term goal. Now that many of the players in this process have been identified, in the next decade, we will most likely see tremendous progress in the development of therapeutic agents that suppress inflammation and promote natural and inducible T_{reg} cell function. Inhibition of tissue inflammation and a simultaneous increase in T_{reg} cell function may induce long-term and lasting resolution of immune-mediated diseases in a tissue-specific and effector-subset-specific manner.

Identification of tissue-specific and subset-specific T_{reg} cells provides a means by which the frequency and function of specific T_{reg} cells can be boosted without inducing generalized immunosuppression, which has marred many of the current therapies that are used in the treatment of autoimmune diseases. A case in point is the antibody to $\alpha 4$ integrin (natalizumab). This antibody inhibits the trafficking of all T cells and effectively inhibits clinical disease in patients with multiple sclerosis and Crohn's disease, but a small percentage of these patients develop progressive multifocal leukoencephalopathy, an infection in the brain that can have lethal or debilitating consequences⁷⁶.

With increased numbers of environmental triggers (for example, smoking, infections, toxins and dysbiosis) being identified, the

development of autoimmune and immune-mediated diseases can be prevented by limiting the exposure of people with a genetic predisposition to autoimmune disease to environmental triggers that initiate disease. With the identification of various microbial species that can actively expand nT_{reg} and iT_{reg} cells not only in the gut but also at distal sites, one can envision actively inducing a shift in gut microbiota to affect immune-mediated diseases and promoting a healthy microbiome to maintain immunologic homeostasis. However, such an endeavor would involve the active collaboration of many investigators in multiple disciplines, including geneticists, immunologists, microbiologists, epidemiologists, clinicians, dietitians and individuals in the pharmaceutical industry, to make disease resolution and prevention a reality.

COMPETING FINANCIAL INTERESTS

The authors declare no competing financial interests.

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