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Regulatory mechanisms of the immune system in multiple sclerosis. T regulatory cells: turned on to turn off

Abstract The immune system is homeostatically regulated to maintain a balance between triggering of inflammatory responses and protecting against self-directed autoimmunity. In autoimmune diseases, such as multiple sclerosis, this balance is disrupted. T regulatory cells, characterised by the expression of the cell surface marker CD25 and the transcription factor FoxP3, play a key role in maintaining this balance by suppression of the activity of effector T cells principally through cell-cell contact. These inhibitory effects of T_{reg} cells appear to be impaired in multiple sclerosis. Facilitating the activity of T_{reg} cells may thus be a promising therapeutic strategy for treatment of multiple sclerosis. Glatiramer acetate stimulates proliferation of $CD4^+CD25^+$ $FoxP3^+$ T_{reg} cells and their transmigration across the blood brain barrier in in vitro models. These T_{reg} cells may cross-react with myelin within the nervous system to turn off encephalitogenic effector T cells. In multiple sclerosis, glatiramer acetate can induce a phenotypic shift in T cell populations towards $CD4^+CD25^+$ T_{reg} cells in vitro, whereas it has been demonstrated in vivo that glatiramer acetate exposure induces a population of $CD8^+$ suppressor T cells that regulate $CD4^+$ T cell proliferation. In addition, glatiramer acetate down-regulates the expression of the Toll-like receptor TLR9, which may be involved in an innate immunity component of autoimmune disease, in a mouse EAE model. Upstream promotion of T_{reg} cell differentiation by blockade of the co-stimulatory molecule CD154 may also be of benefit in preventing autoimmune disease, as illustrated by a promising exploratory Phase I study in multiple sclerosis.

Key words Multiple sclerosis · inflammation · experimental autoimmune encephalomyelitis · T regulatory cells · glatiramer acetate

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Immune system homeostasis and dysfunction in autoimmune disease

In the healthy individual, the immune system is regulated to maintain a balance between triggering of inflammatory responses, notably through activation of populations of effector cells, such as Th1 or Th2 cells, and stasis maintained by regulatory cells. In autoimmune diseases, such as multiple sclerosis, this balance is disrupted when one or more of the several mechanisms that normally maintain homeostasis dysfunctions. An inflammatory state may arise either due to excessive activation or stimulation of effector cells resulting in a hyperintense pro-inflammatory state, or if control of immune tolerance exercised by regulatory cells is insufficient. Several mechanisms cooperate to maintain tolerance and prevent the development of autoimmune disease. These mechanisms include relative T cell inactivity or T cell anergy [3, 9], T cell depletion by apoptosis [8, 26] and active immune suppression [18, 27]. In autoimmune disease, a pro-inflammatory state may develop when one of these mechanisms fails. The therapeutic goal for such diseases is thus to re-establish immune homeostasis and restore the balance between effector and regulatory T lymphocytes. The principal

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mechanisms involved in maintaining immune homeostasis, particularly with respect to the development, course and treatment of multiple sclerosis are reviewed in this article.

Regulation of inflammation

Inflammation is regulated by two populations of T cells, natural (constitutive) T regulatory cells and induced T regulatory cells (Fig. 1). 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. Specific populations of natural T_{reg} cells are generated by interaction with immature antigen-presenting cells (APCs) in the periphery. They recognise 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 T_H1 cells.

Induced T_{reg} cells are derived from naïve $CD8^+$ or $CD4^+$ precursor cells in the thymus in response to the local antigen or cytokine environment. Three subpopulations of induced T_{reg} cells can be distinguished on the basis of the surface markers that they express, namely $CD8^+$ T_{reg} cells, T_H3 cells and TR1 cells, the latter two groups being derived from $CD4^+$ precursors. In autoimmune disease, auto-antigens can stimulate the differentiation of these induced T_{reg} cells.

T_{reg} cells release cytokines such as IL-10 and TGF-β, that suppress the activity of effector T cells as well as of APCs. In addition, effector cells and APCs may be inhibited by direct contact with T_{reg} cells and the interaction of cell surface proteins. The beneficial effect of this inhibitory effect on immune function is to prevent the development of hypersensitivity reactions, of allergies and autoimmune disease, as well as promoting long-term graft tolerance. On the other hand, there may also be detrimental effects of inhibition of immune function by T_{reg} cells, in particular attenuation of immunity to pathogens and reduced immunological surveillance and prevention of tumorogenesis.

A key regulatory molecule in the development and function of T_{reg} cells is FoxP3, a transcriptional repression factor of the FOrkhead/winged box family that is expressed by all functional T_{reg} cells except the T_R1 class. Mutations in FoxP3 impair 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 [4, 5].

Function of natural T regulatory cells in autoimmune disease

A clue to the role of T_{reg} cells has come from experiments in which these cells have been deleted in mice. Such animals readily develop autoimmune diseases and are sensitised to experimental paradigms of autoimmune disease, such as inflammatory bowel disease (IBD) or experimental autoimmune encephalomyelitis (EAE) [19, 20]. The adoptive transfer of naïve $CD4^+CD25^-$ effector T cells obtained from a donor mouse, in whom T_{reg} cells had been previously deleted, into a host athymic mouse results in marked proliferation of the effector T cells and the development of systemic autoimmune disease [21]. In another series of experiments, $CD25^+$ T_{reg} cells were deleted by injection of an anti-CD25 antibody to mice; these animals become more sensitive to EAE induced by inoculation with a synthetic myelin basic protein- (MBP) related peptide (Fig. 1) [22]. In humans, T_{reg} cells expressing CD4, CD25 and Fox3P potently suppress proliferation of $CD4^+CD25^-$ effector T cells upon stimulation in vitro [25]. Suppression of proliferation appeared to be mediated principally by cell-cell interactions, but a modulatory role of cytokines released from $CD4^+CD25^+Fox3P^+$ T_{reg} cells cannot be excluded. Such studies have given rise to the hypothesis that autoimmunity is normally kept under control in healthy individuals by a bystander suppression effect mediated by T_{reg} cells [19].

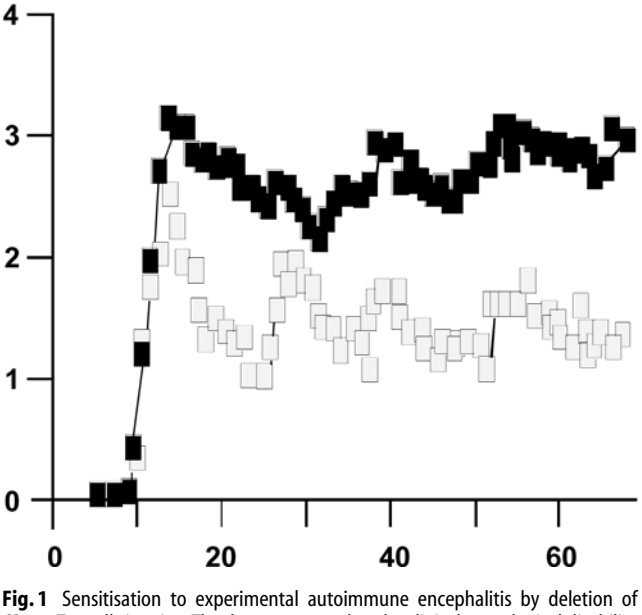

Fig. 1 Sensitisation to experimental autoimmune encephalitis by deletion of $CD25^+$ T_{reg} cells in mice. The data correspond to the clinical neurological disability score in normal mice (open symbols) and in mice in whom $CD25^+$ T_{reg} cells had been deleted with an anti-CD25 antibody (closed symbols). Experimental autoimmune encephalitis was produced by inoculation with a synthetic myelin basic protein-related peptide. Reproduced from [22] with permission.

Bystander suppression by T_{reg} cells can be considered an evolutionary response to counter the potential danger of autoimmunity arising from immune cross-reactivity between xenobiotic and self antigens. Such cross-reactivity is thought to be the initiating event in the activation of autoimmune responses, whereby a population of activated effector T cells is initially generated in response to a xenobiotic antigen and then can be re-activated by host antigens which are recognised by the same T cell receptors. The ability of a given T cell receptor to recognise both xenobiotic and host antigens is due to molecular mimicry between the two antigens.

There is evidence to suggest that immunoregulation by T_{reg} cells in multiple sclerosis is dysfunctional. Although the titres of $CD4^+CD25^{high}$ T_{reg} cells in patients with multiple sclerosis are similar to those of healthy control subjects [6], the capacity of these cell populations to inhibit the activity of encephalitogenic T cells appears to be impaired. In one study, the inhibitory effect of $CD4^+CD25^{high}$ T_{reg} cells from patients with multiple sclerosis on proliferation of effector T cells and on inflammatory cytokine production by these cells was blunted [24]. Another study demonstrated that the inhibition of T cell proliferation in response to myelin oligodendrocyte protein by T_{reg} cells from patients with multiple sclerosis was reduced [6].

Harnessing T regulatory cells to treat autoimmune disease

The bystander suppression hypothesis provides a rationale for treating autoimmune-disease by epitope-specific activation of T_{reg} cells. This approach has been validated in animal models, where it has been demonstrated that expansion of a T_{reg} cell line recognising a proteolipid protein- (PLP) related epitope and passive transfer of these cells to host mice would protect the latter against PLP-evoked EAE [28].

In the central nervous system, neurones also play an important immunoregulatory role and can direct T cell differentiation. For example, in the EAE model, neurones promote the conversion of a population of encephalitogenic $CD4^+CD25^-$ effector T cells to $CD4^+CD25^+FoxP3^+$ T_{reg} cells through a TGF-β-mediated pathway [13]. The resulting T_{reg} cells exert a bystander suppressor effect on encephalitogenic T cells and attenuate the severity of experimental autoimmune encephalomyelitis [13].

Glatiramer acetate (GA) is a mixture of polypeptides composed of four amino-acids that are major constituents of MBP. GA has been shown to suppress EAE in experimental animals and also reduce disease activity in human multiple sclerosis. It has been suggested that GA is effective due to molecular mimicry of an antigenic motif found in MBP [1]. In this context, GA may have the capacity to stimulate the proliferation of GA-specific T_{reg} cells through conditioning of the dendritic cells [2]. These T cells may be cross-reactive with MBP and inhibit encephalitogenic effector T cells in sites of myelin destruction. It has been demonstrated that GA does indeed induce generation and proliferation of T_{reg} cells, as visualised by an increase in expression of FoxP3 in both rodent models of EAE and human multiple sclerosis [7]. In mice, we have observed that the titre of IL-10 may increase in vivo, whereas IFN-α is more pronounced during in vitro generation. TGF-β is now appreciated to be an important switch factor for T_{regs} in both human and murine models. These GA-specific $CD4^+CD25^+FoxP3^+$ T_{reg} cells were subsequently shown to suppress the proliferation of naïve effector T cells in vitro.

In EAE and in human multiple sclerosis, T cells initially activated in the periphery need to infiltrate the central nervous system in order to be reactivated locally by their cognate antigen. Specific mechanisms exist to allow immune cells to leave the circulation and enter the nervous system. In general, leukocytes must undergo an initial rolling interaction on the endothelial cell surface before being activated by a chemoattractant and adhering to the endothelial surface. Adherent leucocytes are then able to leave the vasculature by a process known as transmigration [16]. These mechanisms involve interplay between adhesion molecules (integrins) on the activated leukocyte and their receptors on the endothelial surface, which are up-regulated in response to cytokines released from the leukocytes. The key components involved in immune cell adhesion and migration at the blood brain barrier are α4-integrin (also known as VLA-4) and its endothelial receptor vascular cell adhesion molecule (VCAM)-1.

In vitro transmigration assays can be used to investigate the mechanisms underlying transmigration of cells across the blood–brain barrier. The assay uses a transwell chamber system with a brain-derived endothelial monolayer to simulate the blood–brain barrier. The leukocytes of interest, labelled with 5-chloromethylfluorescein diacetate (CMFDA), a green fluorescent dye, are added to the top compartment and chemoattractants such as IL-2, IL-15 or granulocyte/macrophage colony-stimulating factor (GMCSF) added to the bottom compartment, the other side of the endothelial monolayer. Migration of leukocytes across the monolayer can be visualised by confocal microscopy.

In this model, GA stimulates migration of $CD4^+CD25^+$ cells across the endothelial monolayer in response to IL-2 (Table 1). This effect was selective for this chemoattractant, since migration in response to GMCSF was unaffected. A possible mechanism for this effect would be up-regulation of adhesion molecules. However, GA had no effect on the expression of α4-integrin and antibodies against α4-integrin did not modify the proliferative response to GA. These results suggest

Table 1 Migration of $CD4^+CD25^+$ T_{reg} cells across an endothelial monolayer. Migration was stimulated by addition of GMCSF or interleukin-2 to the transendothelial compartment. GA: glatiramer acetate

Stimulant	Untreated	GA treated
GMCSF	+	+
IL-2	++	++++

that GA enhances migration of T_{reg} cells across the cell monolayer independently of α4-integrin express.

Other immune cell populations may also be induced by GA. For example, specific populations of GA-reactive $CD8^+$ T cells are differentially up-regulated in multiple sclerosis patients treated with GA [10]. For unknown reasons, there is a relative deficit of this class of T cell in multiple sclerosis, and GA treatment thus helps normalise this deficit. $CD8^+$ T cells have suppressor functions and interact with $CD4^+$ T cells by cell-cell contact leading to apoptosis of the $CD4^+$ T cells. $CD8^+$ T cells isolated from GA-treated patients show enhanced cytotoxic activity in response to GA stimulation [23].

In addition to the role of T_{reg} cells in preventing breakthrough of autoimmunity, the innate immune system may also be important in determining susceptibility to autoimmune diseases such as EAE. Of primary importance in innate immune surveillance are the Toll-like receptors (TLR), located on immune cells, typically on antigen-presenting cells (APCs), which recognise characteristic signatures of intracellular (viral and retroviral) or extracellular (most bacterial and fungal) pathogens. These signatures are typically nucleic acid sequences, such as CpG motifs, that are present in the DNA of invading intracellular pathogens, but are not present in host DNA. TLR9 is a member of the Toll-like receptor family expressed primarily in plasmacytoid dendritic cells and B cells [12]. Activation of TLR9 by CpG motifs leads to secretion of pro-inflammatory cytokines such as INF-α which activate TH1 cells and thus lead to an inflammatory attack on invading pathogens.

TLR9-mediated innate immunity appears to play a role in clinical manifestation of disease in the rodent EAE model. For example, increased expression of TLR9 has been observed in mouse EAE, even in the absence of any pathogen-derived CpG activators [17]. In addition, mice in whom expression of MyD88, an obligate signal transduction protein for TLRs, has been suppressed are resistant to standard protocols for induction of EAE [17]. Studies such as this demonstrate that TLR9 is an essential modulator of the autoimmune process during the acute inflammatory phase of EAE. If a method could be developed to attenuate TLR9-mediated innate immunity, then it may be possible to abrogate autoimmune pathology such as that observed in EAE. In this context, the observation that expression of TLR9 in the CNS is reduced in mice treated with glatiramer acetate (Fig. 2) illustrates the feasibility of this approach.

Other potential immunoregulatory targets for the treatment of multiple sclerosis

It may also be possible to facilitate T_{reg} cell function by acting upstream on APCs. When mature APCs interact with naïve T cells, they promote differentiation of T cells towards a T_H1 phenotype and inhibit differentiation towards T_{reg} cells. This differentiating drive depends on interactions between CD40 and CD125 in the APC [15]. Blockade of CD40/CD125 may thus facilitate T_{reg} cell function. For example, treatment of mice with anti-CD125 antibodies increases the number of FoxP3 expressing T cells [14] and promotes allograft tolerance.

An exploratory Phase I study has evaluated treatment with anti-CD154 antibody in patients with multiple sclerosis [11]. Twelve patients received four administrations of escalating doses of anti-CD125 antibody (1–15 mg/kg) over an eight-week period and then GA (six patients), high dose interferon-β, (three patients) or no immunomodulatory treatment (three patients). This treatment was shown to increase the relative proportion of $CD4^+CD25^+$ T_{reg} cells from 4% of all $CD4^+$ T cells to between 24% and 30% at the end of the eight week treatment period. A profound reduction in clinical relapse rate was observed following anti-CD-154 treatment which was maintained over a five-year follow up period. The mean relapse rate in the twelve months preceding treatment was 2.7. No relapses were observed in the year following treatment. Over the following four years, eight relapses were observed overall in the twelve patients. Mean EDSS scores remained stable over the five year period (2.3 ± 0.8 at baseline and 2.5 ± 1.6 after five years).

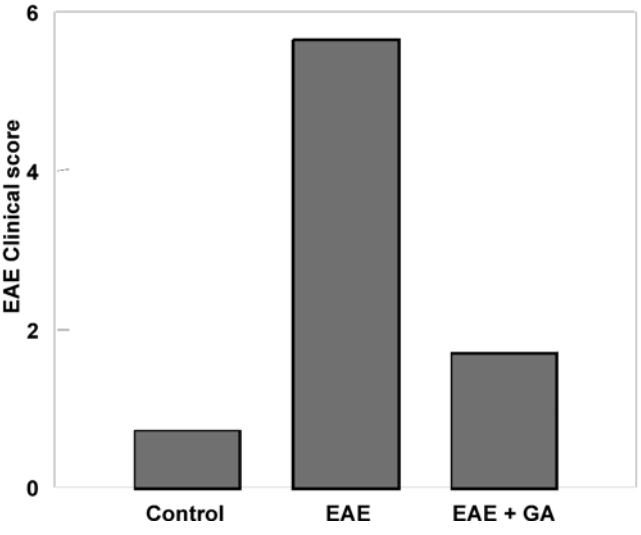

Fig. 2 Clinical expression of experimental autoimmune disease

These effects on relapse rates and EDSS appeared to be dose-related.

Conclusions

T_{reg} cells play an important role in maintaining homeostasis in the immune system so that effective defence against infections is ensured without the development of autoimmune disease. Promoting the proliferation or activity of these T_{reg} cells represents a rational approach for the treatment of autoimmune disease. Such an effect may contribute to the beneficial effects of GA in multiple sclerosis, since GA treatment has been shown to stimulate the proliferation of T_{reg} cells and to facilitate their passage across the blood-brain barrier in experimental models. The results of a pilot clinical study of anti-CD125 antibodies, which stimulate production of T_{reg} cells, in multiple sclerosis also supports the interest of T_{reg} promotion strategies in the treatment of autoimmune disease.

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