



HAL
open science

Immune tolerance and control of CNS autoimmunity: from animal models to MS patients

Cécile Cassan, Roland Liblau

► **To cite this version:**

Cécile Cassan, Roland Liblau. Immune tolerance and control of CNS autoimmunity: from animal models to MS patients. *Journal of Neurochemistry*, 2007, 100 (4), pp.883 - 892. 10.1111/j.1471-4159.2006.04270.x . hal-03095859

HAL Id: hal-03095859

<https://hal.science/hal-03095859>

Submitted on 2 Feb 2021

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

MINI-REVIEW

Immune tolerance and control of CNS autoimmunity: from animal models to MS patients

Cécile Cassan* and Roland S. Liblau*[†]‡

*INSERM, U563, Centre de Physiopathologie de Toulouse-Purpan, Toulouse, France

[†]Immunology Laboratory Toulouse University Hospital, Toulouse, France[‡]Université Paul-Sabatier, Toulouse, France**Abstract**

Multiple sclerosis (MS) is a chronic inflammatory disease resulting in demyelination and axonal loss within the CNS. An autoimmune reaction directed against myelin antigens contributes to the disease process. As the CNS has long been considered an immune privileged site, how such an immune response can develop locally has remained enigmatic. Recent data, mostly based on the study of animal models for MS, have shown that the CNS is in fact more permissive to the development of immune responses than previously thought. This observation is counterbalanced by the fact that immune tolerance to myelin antigens can be induced outside the CNS. This review focuses on the mechanisms preventing CNS autoimmunity, which act in three separate tissues. In the

thymus, expression of CNS autoantigens promotes partial protection, notably through elimination of autoreactive T cells. In the secondary lymphoid organs, the remaining autoreactive T cells are kept under control by the naturally occurring regulatory T cells of the CD4⁺Foxp3⁺ phenotype. In the CNS, multiple mechanisms including the local activation of regulatory T cells further limit autoimmunity. A better understanding of the induction of regulatory T cells, of their mechanisms of action, and of approaches to manipulate them *in vivo* may offer new therapeutic opportunities for MS patients.

Keywords: autoimmunity, central nervous system, multiple sclerosis, regulatory T cells, tolerance.

J. Neurochem. (2007) **100**, 883–892.

Multiple sclerosis (MS) is a chronic demyelinating disease that usually begins in young adulthood. Although MS does not generally reduce life expectancy, irreversible sequelae progressively lead to severe disability in most patients. Because of the increasing prevalence of the disease (Pugliatti *et al.* 2001), its chronic course, and the only partial effect of current disease-modifying drugs, MS has major social and economical consequences. The histopathology of MS is characterized by multifocal inflammation, demyelination and glial scar formation in the white matter of the CNS, which lead to axonal damage. The pathophysiology of the disease appears heterogeneous and has yet to be fully understood. However, demyelination in MS is mediated by an inflammatory response characterized by the recruitment of CD4⁺ and CD8⁺ T cells as well as macrophages. T and B cells specifically directed against myelin self-antigens, such as myelin basic protein (MBP) (Bielekova *et al.* 2000; Berger *et al.* 2003; Bielekova *et al.* 2004), proteolipid protein (PLP; Bielekova *et al.* 2004) and myelin oligodendrocyte glycoprotein (MOG; Olsson *et al.* 1992; Genain *et al.* 1999),

contribute to this deleterious immune response [for an exhaustive review, refer to Sospedra and Martin (2005)]. The triggers that first cause the immune system to attack myelin are largely unknown. Multiple genes including the HLA locus, which encodes for molecules involved in the presentation of antigenic peptides to T cells, confer disease susceptibility very likely through variable combinations. Moreover, the role of microbial, in particular viral, agents has long been hypothesized. Some clinical and histological

Received July 20, 2006; revised manuscript received September 21, 2006; accepted October 2, 2006.

Address correspondence and reprint requests to Roland S. Liblau, INSERM U563 CPTP, Bât.B 1er étage, CHU Purpan BP 3028, 31024 Toulouse Cedex 3, France. E-mail: rolandliblau@hotmail.com

Abbreviations used: AIRE, autoimmune regulator; EAE, experimental autoimmune encephalomyelitis; IRBP, interphotoreceptor retinoid-binding protein; MBP, myelin basic protein; MHC, major histocompatibility complex; MOG, myelin oligodendrocyte glycoprotein; MS, multiple sclerosis; NK, natural killer; PLP, proteolipid protein; TCR, T cell receptor; Tregs, CD4⁺Foxp3⁺ regulatory T cells.

aspects of the disease are mimicked in experimental autoimmune encephalomyelitis (EAE), an animal model for MS induced by immunization with myelin self-antigens in adjuvant (Kuchroo *et al.* 2002). EAE has been central for the development of several therapeutic approaches that are currently approved for MS, underlining the relevance of this model (Zamvil and Steinman 2003).

Important advances have been made recently on the immune mechanisms involved in CNS tissue damage, both in EAE and MS. This review focuses on major progress in the field of immune tolerance to CNS self-antigens, and in particular on the role of the thymus and the thymus-derived natural regulatory T cells (Treg). Indeed, this new knowledge has important implications for understanding the pathophysiology of MS and developing new therapeutic strategies.

Is the CNS really an immunologically privileged organ?

The CNS has long been considered a privileged organ for induction of immune responses, as allografts and tumours are less readily eliminated in the CNS compared with other tissues. This protection from CNS immune pathology is thought to be essential owing to the limited renewal and the post-mitotic nature of neurons. According to the classical view, several mechanisms concur to establish this immune privilege. First, the blood–brain barrier was shown to prevent trafficking of resting lymphocytes into the CNS while allowing entry of recently activated cells (Hickey *et al.* 1991). Second, it was assumed that immune responses could not develop in the CNS because only few resident cells constitutively express major histocompatibility complex (MHC) molecules in the steady state, and because the CNS was thought to be devoid of professional antigen-presenting cells [for review see Perry (1998)]. Third, local tolerogenic mechanisms exist within the CNS. Specifically, CNS expression of Fas-ligand, prostaglandin E₂, TGF- β , and galectin-9 has been associated with functional silencing or killing of incoming T cells (Khoury *et al.* 1992; Mannie *et al.* 1995; Issazadeh *et al.* 1998; Suvannavejh *et al.* 2000; Zhu *et al.* 2005; Liu *et al.* 2006b).

However, several lines of evidence now indicate that the immune privilege of the CNS is not foolproof. First, access of immune cells to the CNS is limited but not forbidden. Indeed, naïve T cells have been shown to traffic to the inflamed brain (Krakowski and Owens 2000; Aloisi and Pujol-Borrell 2006). Even more perplexing, studies on T-cell migration in the steady state in mice strongly suggest that both naïve CD4⁺ and CD8⁺ T cells are able to patrol non-lymphoid tissues, including the CNS (Brabb *et al.* 2000; Cose *et al.* 2006). However, while naïve T cells can circulate in the CNS without triggering a harmful response, activation of myelin-specific T cells is not always sufficient to allow their entry, which needs additional signals such as those

delivered by specific microbial components through the Toll-like receptors (TLR; Brabb *et al.* 1997; Waldner *et al.* 2004).

Second, antigen presentation does occur in the CNS. Indeed, upon exposure to a pro-inflammatory environment, oligodendrocytes and neurons express MHC class I molecules (allowing antigen presentation to CD8⁺ T cells), while astrocytes and microglial cells can express both MHC class I and II molecules (allowing antigen presentation to both CD8⁺ and CD4⁺ T cells). Moreover, it has been shown that dendritic-like cells are necessary and sufficient to reactivate CD4⁺ T cells within the CNS, in a passive model of EAE (Greter *et al.* 2005). In addition, CNS dendritic cells are able to initiate epitope spreading (McMahon *et al.* 2005), a phenomenon whereby immune reactivity is induced against additional self-epitopes during chronic tissue inflammation (McRae *et al.* 1995; Tuohy *et al.* 1999). Importantly, vessel-associated dendritic cells have also been found in active MS lesions (Kivisakk *et al.* 2004; Greter *et al.* 2005). The presence of dendritic cells in the inflamed CNS has important implications for MS pathogenesis, as it indicates that reactivation or even priming of incoming T cells is possible within the CNS.

Finally, TGF- β long known to exhibit regulatory properties is now being incriminated in pathogenic processes. Indeed, contingent upon an inflammatory cytokine environment, TGF- β also promotes the differentiation of CD4⁺ T cells into a newly identified pathogenic T-cell lineage characterized by IL-17 secretion, referred to as Th-17 cells (Langrish *et al.* 2005; Bettelli *et al.* 2006).

In summary, although under steady-state conditions the CNS is not favourable for development of immune responses, new knowledge indicates that, under inflammatory conditions, T-cell responses can develop within this tissue but are under elaborate control (Fig. 1). To initiate CNS tissue damage, autoreactive T cells must escape control mechanisms that may regulate their peripheral activation, their entry into the CNS, and/or their local reactivation.

Tolerance to CNS autoantigens: a new role for the thymus

Drastic selection of the developing T cells in the thymus is a key step in the prevention of autoimmunity (Kyewski and Klein 2006). Indeed, during thymic ontogeny, nascent T cells expressing T-cell receptor (TCR) that form high-affinity interactions with self-antigens undergo apoptosis (Siggs *et al.* 2006). It has been assumed that this process, called thymic negative selection, eliminates T cells recognizing ubiquitous or blood-borne self-antigens, but spares thymocytes specific for the secluded CNS-restricted self-antigens. However, in recent years, it has been shown that numerous so-called tissue-restricted self-antigens are actually expressed in the normal thymus, and can thereby induce T-cell tolerance. A dedicated subset of thymic antigen-presenting

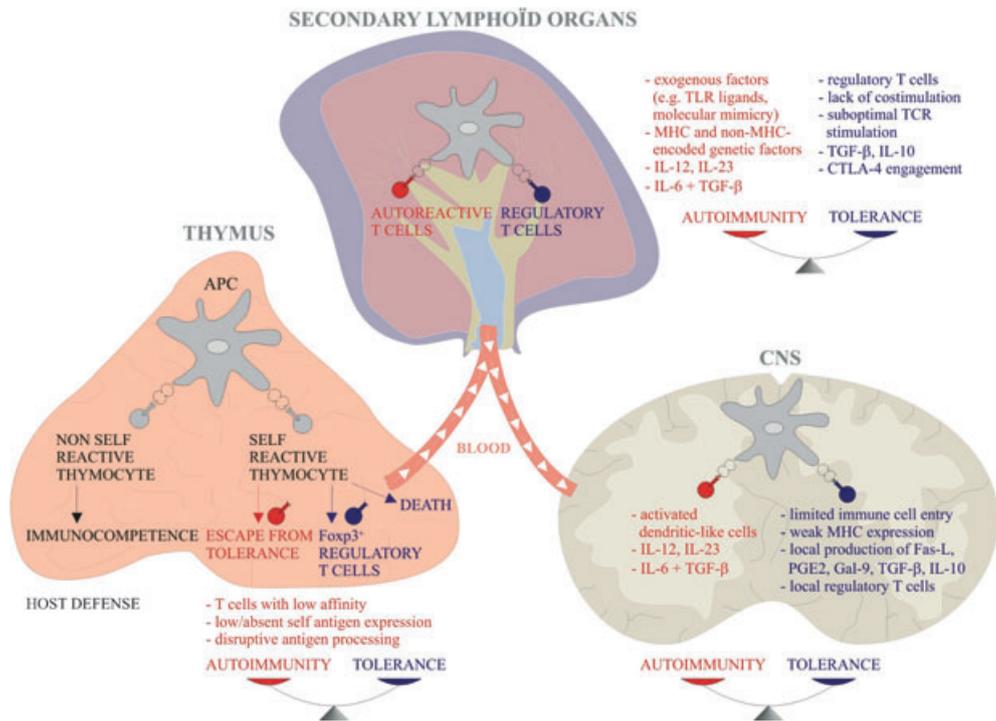


Fig. 1 Schematic description of the events promoting or preventing CNS autoimmunity. The development of CNS autoimmunity results from an imbalance between tolerogenic and activating signals on autoreactive T cells. Those signals can be delivered as early as the thymic ontogeny of autoreactive T cells, resulting in their exit from the thymus, in their demise, or in their differentiation into regulatory T cells. Autoreactive T cells, during their traffic through the secondary lymphoid organs, can also be subjected to triggers, which can lead to

radically different outcomes. Once activated in the lymphoid organs, autoreactive T cells gain access to the CNS and may be locally reactivated and initiate tissue inflammation, or are locally inactivated through a variety of mechanisms. Therefore, multiple physiological control mechanisms operate in different tissues to counteract CNS autoimmunity. These mechanisms could be exploited to design new therapeutic approaches.

cells – the cortical and medullary thymic epithelial cells – mostly carries out this task of expressing a large array of self-antigens both in mice and humans (Derbinski *et al.* 2001). The thymic expression of tissue-restricted antigens is probably under the control of several transcription factors, including the autoimmune regulator (AIRE). AIRE mutation results in incomplete silencing of autoreactive T cells in the thymus, and in development of multiorgan autoimmunity (sparing the CNS) both in mice and men (Peterson *et al.* 1998; Anderson *et al.* 2002; Liston *et al.* 2003; Liston *et al.* 2004).

A variety of CNS self-antigens that are relevant for MS pathogenesis are also expressed in the thymus. Indeed, MBP mRNA and protein are synthesized by several thymic cell types including macrophages (Feng *et al.* 2000; Liu *et al.* 2001). Studies comparing the development of MBP-specific T cells in MBP^{+/+} versus MBP^{-/-} mice have clearly shown that strong negative selection takes place in the thymus of MBP-expressing mice (Huseby *et al.* 2001; Perchellet *et al.* 2004). Interestingly, bone marrow-derived cells rather than thymic epithelial cells are involved in this process (Huseby *et al.* 2001). Additionally, an increased thymic expression of

several MBP isoforms is associated with resistance to the development of MBP-induced EAE in mice (Liu *et al.* 2001). Despite these thymic tolerance mechanisms, potentially harmful MBP-specific T cells are nevertheless circulating in both mice and humans (Kuchroo *et al.* 2002; Sospedra and Martin 2005). Their proportion and functional avidity may, however, vary depending on the extent of thymic MBP expression.

Full-length PLP is barely expressed in murine and human thymus, while DM20, a splice variant lacking the amino acids 115–151 is consistently detected (Pribyl *et al.* 1996; Anderson *et al.* 2000; Klein *et al.* 2000). This expression is mainly attributed to cortical and medullary thymic cells (Klein *et al.* 2000; Derbinski *et al.* 2001). In SJL mice, which are highly susceptible to PLP-induced EAE, encephalitogenic CD4⁺ T cells are primarily specific for PLP_{139–151}, a peptide encoded by the exon of *PLP* not transcribed in the thymus (Anderson *et al.* 2000). Thymus grafting experiments using either PLP-sufficient or PLP-deficient donor mice have unambiguously shown that tolerance of PLP-specific T cells is induced by thymic stromal cells expressing PLP (Klein *et al.* 2000). Similarly, introduction of

PLP_{139–151} peptide in the thymus is sufficient to induce tolerance to this peptide (Anderson *et al.* 2000). In summary, there is a potent induction of tolerance towards the PLP peptides expressed in the thymus.

MOG represents less than 1% of CNS myelin proteins but, however, appears to be a preferential immunologic target in EAE and in MS patients (Kerlero de Rosbo *et al.* 1993; Adelman *et al.* 1995). It therefore became relevant to assess whether MOG was being expressed at all in the thymus. Contrasting with data available for MBP or PLP, the expression of MOG mRNA in the whole thymus is either minimal (Delarasse *et al.* 2003) or absent (Bruno *et al.* 2002). However, using sensitive RT-PCR approaches on purified thymic cell populations, expression of MOG was detected specifically in medullary epithelial cells both in mice (Derbinski *et al.* 2001) and humans (Gotter *et al.* 2004). Studies are currently assessing whether this MOG expression affects T-cell development. Initial results have indicated some degree of tolerance to MOG in MOG^{+/+} versus MOG^{-/-} mouse (Linares *et al.* 2003). However, independent data comparing MOG-specific T cell responses in MOG^{-/-} vs. MOG^{+/+} mice indicate that its expression in the thymus is not sufficient to induce detectable tolerance (Delarasse *et al.* 2003; Fazilleau *et al.* 2006).

Thymic expression of CNS-restricted antigens extends beyond myelin/oligodendrocyte antigens. As an example, S100 β , which is synthesized by astrocytes in the CNS, is detected in the thymus of Lewis rats (Kojima *et al.* 1997) as well as in human medullary and cortical thymic epithelial cells (Bruno *et al.* 2002). This thymic expression is not able to eliminate all S100 β -specific CD4⁺ T cells, as some can be isolated from normal adult rat thymus. Moreover, transfer of these cells following an *in vitro* activation step causes an inflammatory response in the CNS of recipient animals (Kojima *et al.* 1997). Another example is α B-crystallin, a heat-shock protein expressed in astrocytes and oligodendrocytes and a potential target of autoreactive T cells in MS. Its expression in rat thymus is associated with unresponsiveness of peripheral lymphocytes to autologous α B-crystallin (van Stipdonk *et al.* 2000).

Thus, although expression of myelin or astrocyte antigens in the thymus seems to induce negative selection, specific T cells with low avidity or recognizing particular epitopes are spared (Fig. 1). These self-reactive T cells can be found in the periphery of healthy individuals and are likely implicated in the pathogenesis of MS. It is therefore essential that other mechanisms, which may be overridden in MS patients, keep these CNS-specific T cells under control in the secondary lymphoid organs.

Regulatory T cells and CNS autoimmunity

Several T-cell populations contribute to the maintenance of self-tolerance by inhibiting activation of autoreactive

lymphocytes in the lymphoid tissue or/and in the peripheral organs. Among these, regulatory T cells (Tregs) of the CD4⁺CD25⁺Foxp3⁺ phenotype are the most studied and best characterized (Sakaguchi *et al.* 2006). CD4⁺CD25⁺Foxp3⁺ Tregs are generated in the thymus where the transcription factor Foxp3 plays a key role in their development (Fontenot *et al.* 2005; Ziegler 2006). The current concept is that developing thymocytes with intermediate avidity for self-antigens preferentially express Foxp3 and commit to the Treg lineage (Jordan *et al.* 2001; Romagnoli *et al.* 2002; Hsieh *et al.* 2004). Tregs also appear more resistant to negative selection than conventional CD4⁺ T cells, so that upon thymic expression of self-antigen, the balance between self-reactive effector T cells and Tregs is shifted towards tolerance (Jordan *et al.* 2001; Liston *et al.* 2004; van Santen *et al.* 2004). In mice, Foxp3 expression is virtually restricted to CD4⁺CD25⁺ Tregs and therefore represents a reliable nuclear marker for these cells (Fontenot *et al.* 2005). In human, although Foxp3 is transiently expressed in activated CD4⁺ and CD8⁺ non-regulatory T cells, it represents the best marker to date for the Treg population [for review see Ziegler (2006)].

Tregs represent 5–10% of rodent CD4⁺ T cells and 3–9% of human circulating CD4⁺ T cells (Seddiki *et al.* 2006). They control the activation of conventional CD4⁺ T cells, but also CD8⁺ T cells, B lymphocytes, natural killer (NK) and NKT cells, and their precise mechanisms of action are still poorly understood (Azuma *et al.* 2003; Sakaguchi 2004; Ghiringhelli *et al.* 2005; Lim *et al.* 2005). Tregs have been implicated in numerous physiologic or pathologic situations, including control of several autoimmune diseases in animal models as well as in humans (Baecher-Allan and Hafler 2004; Sakaguchi 2004).

Importance of Tregs in EAE and MS

The potential for Tregs to control CNS autoimmunity has been well documented in experimental models. Tregs have been shown to accumulate within the CNS during the recovery from EAE (McGeachy *et al.* 2005). These Tregs isolated from the CNS are highly suppressive *in vitro* and their transfer in low numbers reduces EAE scores of the recipients. Moreover, transfer of large numbers of polyclonal Tregs from unimmunized mice allows reducing EAE severity in C57BL/6 (Kohm *et al.* 2002) or SJL recipients (Zhang *et al.* 2004). Similarly, in a spontaneous model of EAE, obtained in Rag^{-/-} MBP-TCR transgenic mice, transfer of CD4⁺ or CD4⁺CD25⁺ T cells from wild-type animals greatly reduces disease incidence and severity (Olivares-Villagomez *et al.* 1998; Hori *et al.* 2002). Conversely, depletion or inactivation of Tregs by injection of an anti-CD25 monoclonal antibody prior to EAE induction results in heightened activation of autoaggressive T cells. As a consequence, EAE is aggravated (Montero *et al.* 2004; Stephens *et al.* 2005; Cassan *et al.* 2006) and the recovery phase delayed or

abrogated (McGeachy *et al.* 2005; Stephens *et al.* 2005). Depletion of Tregs after the acute phase of EAE renders C57BL/6 mice susceptible to re-induction of disease, to which they are typically resistant (McGeachy *et al.* 2005). In relapsing–remitting EAE models, not only does depletion of Tregs increase acute-phase severity, but it also prevents secondary remissions, documenting the influence of Tregs on disease progression (Gartner *et al.* 2006; Zhang *et al.* 2006).

Given their key function in EAE, it became important to assess whether quantitative or qualitative abnormalities of Tregs existed in MS. No quantitative defects of Tregs, identified on the basis of CD4 and CD25 expression, have been detected in the blood of MS patients relative to healthy controls (Viglietta *et al.* 2004; Haas *et al.* 2005; Huan *et al.* 2005; Venken *et al.* 2006). Moreover, the proportion of Tregs appears similar in peripheral blood and cerebrospinal fluid of MS patients (Haas *et al.* 2005). However, functionally, blood-purified Tregs from relapsing–remitting MS patients display reduced suppressive activity *in vitro* (Viglietta *et al.* 2004; Haas *et al.* 2005; Huan *et al.* 2005). This reduced suppressive activity is intrinsic to Tregs from MS patients and not as a result of a higher activation status or resistance to suppression by their conventional T cells. This functional defect of Tregs is associated with a decrease in Foxp3 mRNA and protein expression among peripheral blood CD4⁺CD25⁺ T cells from MS patients compared with healthy controls (Huan *et al.* 2005). It is not yet clear whether this reduction in Foxp3 expression is because of a lower frequency of Tregs among CD4⁺CD25⁺ T cells or to decreased expression at the cell level. Unexpectedly, the defective suppressive activity of Tregs from relapsing–remitting MS patients does not correlate with occurrence of relapses (Haas *et al.* 2005). Interestingly, contrasting with observations made in patients with relapsing–remitting MS, patients with secondary–progressive MS display normal levels of Foxp3 expression among CD4⁺CD25^{high} T cells, and normal suppression of Tregs *in vitro* (Venken *et al.* 2006). The origin of the global impairment of suppressive function of Tregs in relapsing–remitting MS patients has not yet been elucidated.

Thymic expression of self-antigens and antigen specificity of Tregs: lessons from animal models

As noted above, expression of a self-antigen in the thymus has been shown to reduce the ratio between conventional T cells and Tregs specific for this given antigen (Anderson and Kuchroo 2003; Kyewski and Klein 2006). The study of interphotoreceptor retinoid-binding protein (IRBP), a retinal antigen that represents a prominent target in autoimmune uveitis, provides an illustrative example. Tregs able to control IRBP-specific immune responses following immunization in incomplete Freund's adjuvant are present in IRBP^{+/+} but not in IRBP^{-/-} mice. However, IRBP-deficient mice generate Tregs inhibiting responses against arrestin, another retinal antigen (Grajewski *et al.* 2006). These data indicate that the

presence of a self-antigen is mandatory for the development and/or maintenance of Tregs specific for this antigen. Whether this induction is the result of direct interaction in the thymus between developing T cells and the self-antigen has been recently explored for CNS antigens. For example, the higher susceptibility of SJL mice to EAE compared with the B10.S strain has been related to a lower frequency of Tregs specific for PLP_{139–151}, and to lower thymic expression of PLP in SJL mice (Reddy *et al.* 2004). These data support, but do not formally validate, the hypothesis that a higher level of thymic expression of PLP promotes the generation of PLP-specific Tregs. We have recently developed a mouse model in which a transgenic astroglial self-antigen is also expressed in the thymus. This low level of expression is responsible for the induction and/or expansion of a large population of glial antigen-specific CD4⁺CD25⁺Foxp3⁺ thymocytes (Cabarrocas *et al.* 2006).

Thus, the thymus plays a major role for immune tolerance towards CNS-restricted self-antigen by at least two mechanisms, namely negative selection and induction of Tregs (Fig. 1). This has direct impact on the susceptibility to organ-specific autoimmune diseases, as previously noted for EAE, and emerging data indicate that this also applies to humans.

How to manipulate Tregs in order to control CNS autoimmunity

Because the function of which Tregs has been shown to decrease with age (Tsaknaridis *et al.* 2003; Venken *et al.* 2006), and because of the difficulties to purify them in large quantities, several laboratories have set up protocols to promote their expansion *in vitro*. These have helped the study of their biologic properties *in vitro*, and test their usefulness in cell-based therapy approaches.

Although naturally hypo-responsive, Tregs can be polyclonally expanded upon stimulation with anti-CD3 and anti-CD28 monoclonal antibodies and high amount of exogenous IL-2 or IL-4 (Thornton *et al.* 2004). However, expansion of Tregs specific for a given antigen is best achieved with antigen-loaded dendritic cells (Yamazaki *et al.* 2003; Fehervari and Sakaguchi 2004; Fisson *et al.* 2006). Expanded antigen-specific Tregs are far more efficient than polyclonal Tregs to control autoimmunity (Tang *et al.* 2004; Tarbell *et al.* 2004; Masteller *et al.* 2005).

Another strategy aims at using defined culture conditions to convert conventional CD4⁺ T cells into Tregs *in vitro*. Conversion rate seems maximal in cultures performed with low doses of antigen and immature dendritic cells (Kretschmer *et al.* 2005). Converging data from several laboratories have highlighted the importance of TGF-β for efficient switching of conventional CD4⁺ T cells into Tregs (Chen *et al.* 2003; Kretschmer *et al.* 2005; Weber *et al.* 2006). Moreover, a recent study reports that *in vitro* co-culture of murine neurons and encephalitogenic CD4⁺ T-cell line

converts them into Tregs, which are able to control EAE upon adoptive transfer (Liu *et al.* 2006b). This conversion appears to rely on neuronal expression of both TGF- β and the co-stimulatory CD80/CD86 molecules. These results lead to the hypothesis that neurons themselves can produce factors favouring the development of a protective response *in vivo*, and that encephalitogenic T cells can be turned into Tregs despite their highly differentiated status.

Several groups have attempted to convert conventional CD4⁺ T cells into Tregs by forced expression of the transcription factor Foxp3. Mouse CD4⁺ T cells transduced with a retroviral vector encoding Foxp3 acquire regulatory properties and can display protective function in models of autoimmunity. However, these successes are not universal as Foxp3-transduced PLP_{139–151}-specific CD4⁺ cells still exhibit encephalitogenic properties after transfer into Rag^{-/-} mice (Bettelli *et al.* 2005).

Based on these promising results, a large effort is being devoted to the translation of these protocols to the human situation, which is beginning to bear fruit. Indeed, expansion of human natural Tregs has recently been achieved based on the selection of cells expressing high levels of CD25 and low levels of CD127 (Liu *et al.* 2006a). Moreover, conversion of polyclonal as well as antigen-specific human CD4⁺ T cells into Tregs has also been achieved (Grossman *et al.* 2004; Walker *et al.* 2005). However, some laboratories have failed to recover suppressive activity from Foxp3-transduced human cells despite strong Foxp3 expression (Allan *et al.* 2005).

An alternative to the demanding cellular therapy would be to directly induce or expand Tregs *in vivo*. This possibility is supported by the observation that Tregs proliferate vigorously *in vivo* upon encounter with their antigen (Fisson *et al.* 2003; Walker *et al.* 2003; Apostolou and von Boehmer 2004). Moreover, certain modalities of antigen administration, such as the mucosal route (Chen *et al.* 1994), or the use of a low dose of antigen (Apostolou and von Boehmer 2004; Kretschmer *et al.* 2005), favour the generation of CD4⁺ T cells exhibiting regulatory properties. In addition, several treatments of mice with compounds that proved efficacious in EAE have been shown to induce T-cell populations with properties reminiscent of those of natural Tregs. For example, the mode of action of Copaxone, an approved drug in MS, seems to include, among others, induction of Tregs. Indeed, it has been shown that injection of copolymers promotes the expansion of Tregs in mice (Stern *et al.* 2004) and humans (Hong *et al.* 2005). A potent immune suppressor, LF 15-0195, which has also demonstrated therapeutic efficacy in a rat model of EAE, reduces encephalitogenicity and up-regulates expression of Foxp3 of CD4⁺ T cells from treated rats (Duplan *et al.* 2006). Oral treatment of mice with anti-CD3 monoclonal antibodies prevents the development of EAE or suppresses ongoing EAE, by inducing a population of regulatory cells (Ochi

et al. 2006). Lastly, a high proliferation of Tregs *in vivo*, together with increased regulatory properties, has been achieved by treatment of rats with agonistic anti-CD28 monoclonal antibodies, thereby preventing and even treating EAE in these animals (Beyersdorf *et al.* 2005; Tischner *et al.* 2006).

Tregs: a target for MS immunotherapy?

Several obstacles will have to be resolved before Tregs can be used as therapeutics. The first limitation concerns the antigenic specificity of Tregs. Indeed, although transfer of polyclonal Tregs could alleviate the severity of EAE (Kohm *et al.* 2004), other laboratories have failed to reproduce these data (Mekala *et al.* 2005). Moreover, the fine specificity of the injected Tregs appears to have an impact on efficacy, as PLP_{139–151}-specific Tregs are able to control PLP_{139–151}-induced EAE but not MBP_{87–99}-induced EAE (Yu *et al.* 2005). However, when pre-activated *in vitro* prior to injection, PLP_{139–151}-specific Tregs suppress both PLP_{139–151} and MBP_{87–99}-induced EAE, possibly as a result of their bystander effect. This notion of specificity may pose a problem for the use of Tregs for the treatment of human diseases such as MS. Indeed, the auto-antigen(s) targeted by the pathogenic T cells may vary between patients and even in a given individual along the course of the disease.

The second limitation is the identification of the best strategy to target Tregs. Indeed, autologous cellular therapy using antigen-specific Tregs is a time-consuming and expensive approach that has to be tailored for each treated patient. However, *in vivo* induction or amplification of Tregs by injection of autoantigens could worsen the disease by enhancing the deleterious immune response.

Another obstacle inherent to humans is that the treatment has to block an ongoing disease process. Indeed, Tregs do not function optimally in an inflammatory milieu (Pasare and Medzhitov 2003). As a consequence, combined protocols using both promotion of Tregs and inactivation of pathogenic lymphocytes might well be necessary for optimal efficiency. The identification of molecules, such as rapamycin, that preferentially promote expansion of Tregs while inhibiting pathogenic lymphocytes, opens up new possibilities (Battaglia *et al.* 2006).

To conclude, thymus-derived or peripherally induced Tregs have strong potential in controlling the deleterious CNS immune response in MS. The current aim is to optimize the *ex vivo* or *in vivo* approaches to selectively induce and/or expand CNS-specific human Tregs from the polyclonal T-cell repertoire. Once activated and expanded in the lymphoid tissue, these Tregs will migrate to the inflamed CNS and be reactivated by local dendritic cells. Because of their bystander suppressive capacity, they will dampen the local pathogenic immune response regardless of its fine antigenic specificity. Such an optimistic scenario, however, needs to be

rigorously challenged before potential application in the clinic.

Acknowledgements

The authors thank the French MS society (ARSEP), INSERM, the European Union (NeuroProMiSe), and the Region Midi-Pyrénées for supporting their work, and Drs A. Saoudi and D. Gonzalez-Dunia for helpful comments on the manuscript.

References

- Adelmann M., Wood J., Benzel I., Fiori P., Lassmann H., Matthieu J. M., Gardinier M. V., Dormmair K. and Linington C. (1995) The N-terminal domain of the myelin oligodendrocyte glycoprotein (MOG) induces acute demyelinating experimental autoimmune encephalomyelitis in the Lewis rat. *J. Neuroimmunol.* **63**, 17–27.
- Allan S. E., Passerini L., Bacchetta R., Crellin N., Dai M., Orban P. C., Ziegler S. F., Roncarolo M. G. and Levings M. K. (2005) The role of two FOXP3 isoforms in the generation of human CD4⁺ Tregs. *J. Clin. Invest.* **115**, 3276–3284.
- Aloisi F. and Pujol-Borrell R. (2006) Lymphoid neogenesis in chronic inflammatory diseases. *Nat. Rev. Immunol.* **6**, 205–217.
- Anderson A. C. and Kuchroo V. K. (2003) Expression of self-antigen in the thymus: a little goes a long way. *J. Exp. Med.* **198**, 1627–1629.
- Anderson A. C., Nicholson L. B., Legge K. L., Turchin V., Zaghoulani H. and Kuchroo V. K. (2000) High frequency of autoreactive myelin proteolipid protein-specific T cells in the periphery of naive mice: mechanisms of selection of the self-reactive repertoire. *J. Exp. Med.* **191**, 761–770.
- Anderson M. S., Venanzi E. S., Klein L. *et al.* (2002) Projection of an immunological self shadow within the thymus by the Aire protein. *Science* **298**, 1395–1401.
- Apostolou I. and von Boehmer H. (2004) *In vivo* instruction of suppressor commitment in naive T cells. *J. Exp. Med.* **199**, 1401–1408.
- Azuma T., Takahashi T., Kunisato A., Kitamura T. and Hirai H. (2003) Human CD4⁺ CD25⁺ regulatory T cells suppress NKT cell functions. *Cancer Res.* **63**, 4516–4520.
- Baecher-Allan C. and Hafler D. A. (2004) Suppressor T cells in human diseases. *J. Exp. Med.* **200**, 273–276.
- Battaglia M., Stabilini A., Draghici E., Migliavacca B., Gregori S., Bonifacio E. and Roncarolo M. G. (2006) Induction of tolerance in type 1 diabetes via both CD4⁺CD25⁺ T regulatory cells and T regulatory type 1 cells. *Diabetes* **55**, 1571–1580.
- Berger T., Rubner P., Schautzer F., Egg R., Ulmer H., Mayringer I., Dilitz E., Deisenhammer F. and Reindl M. (2003) Anti-myelin antibodies as a predictor of clinically definite multiple sclerosis after a first demyelinating event. *N. Engl. J. Med.* **349**, 139–145.
- Bettelli E., Dastrange M. and Oukka M. (2005) Foxp3 interacts with nuclear factor of activated T cells and NF- κ B to repress cytokine gene expression and effector functions of T helper cells. *Proc. Natl Acad. Sci. USA* **102**, 5138–5143.
- Bettelli E., Carrier Y., Gao W., Korn T., Strom T. B., Oukka M., Weiner H. L. and Kuchroo V. K. (2006) Reciprocal developmental pathways for the generation of pathogenic effector TH17 and regulatory T cells. *Nature* **441**, 235–238.
- Beyersdorf N., Gaupp S., Balbach K., Schmidt J., Toyka K. V., Lin C. H., Hanke T., Hunig T., Kerkau T. and Gold R. (2005) Selective targeting of regulatory T cells with CD28 superagonists allows effective therapy of experimental autoimmune encephalomyelitis. *J. Exp. Med.* **202**, 445–455.
- Bielekova B., Goodwin B., Richert N. *et al.* (2000) Encephalitogenic potential of the myelin basic protein peptide (amino acids 83–99) in multiple sclerosis: results of a phase II clinical trial with an altered peptide ligand. *Nat. Med.* **6**, 1167–1175.
- Bielekova B., Sung M. H., Kadom N., Simon R., McFarland H. and Martin R. (2004) Expansion and functional relevance of high-avidity myelin-specific CD4⁺ T cells in multiple sclerosis. *J. Immunol.* **172**, 3893–3904.
- Brabb T., Goldrath A. W., von Dassow P., Paez A., Liggitt H. D. and Gorman J. (1997) Triggers of autoimmune disease in a murine TCR-transgenic model for multiple sclerosis. *J. Immunol.* **159**, 497–507.
- Brabb T., von Dassow P., Ordonez N., Schnabel B., Duke B. and Gorman J. (2000) *In situ* tolerance within the central nervous system as a mechanism for preventing autoimmunity. *J. Exp. Med.* **192**, 871–880.
- Bruno R., Sabater L., Sospedra M., Ferrer-Francesch X., Escudero D., Martinez-Caceres E. and Pujol-Borrell R. (2002) Multiple sclerosis candidate autoantigens except myelin oligodendrocyte glycoprotein are transcribed in human thymus. *Eur. J. Immunol.* **32**, 2737–2747.
- Cabarrocas J., Cassan C., Magnusson F. *et al.* (2006) Foxp3⁺ CD25⁺ regulatory T cells specific for a neo-self-antigen develop at the double-positive thymic stage. *Proc. Natl Acad. Sci. USA* **103**, 8453–8458.
- Cassan C., Piaggio E., Zappulla J. P., Mars L., Couturier N., Bucciarelli F., Desbois S., Bauer J., Gonzalez-Dunia D. and Liblau R. (2006) Pertussis toxin reduces the number of splenic Foxp3⁺ regulatory T cells. *J. Immunol.* **177**, 1552–1560.
- Chen W., Jin W., Hardegen N., Lei K. J., Li L., Marinos N., McGrady G. and Wahl S. M. (2003) Conversion of peripheral CD4⁺CD25⁺ naive T cells to CD4⁺CD25⁺ regulatory T cells by TGF- β induction of transcription factor Foxp3. *J. Exp. Med.* **198**, 1875–1886.
- Chen Y., Kuchroo V. K., Inobe J., Hafler D. A. and Weiner H. L. (1994) Regulatory T cell clones induced by oral tolerance: suppression of autoimmune encephalomyelitis. *Science* **265**, 1237–1240.
- Cose S., Brammer C., Khanna K. M., Masopust D. and Lefrancois L. (2006) Evidence that a significant number of naive T cells enter non-lymphoid organs as part of a normal migratory pathway. *Eur. J. Immunol.* **36**, 1423–1433.
- Delarasse C., Daubas P., Mars L. T. *et al.* (2003) Myelin/oligodendrocyte glycoprotein-deficient (MOG-deficient) mice reveal lack of immune tolerance to MOG in wild-type mice. *J. Clin. Invest.* **112**, 544–553.
- Derbinski J., Schulte A., Kyewski B. and Klein L. (2001) Promiscuous gene expression in medullary thymic epithelial cells mirrors the peripheral self. *Nat. Immunol.* **2**, 1032–1039.
- Duplan V., Beriou G., Heslan J. M., Bruand C., Dutartre P., Mars L. T., Liblau R. S., Cuturi M. C. and Saoudi A. (2006) LF 15–0195 treatment protects against central nervous system autoimmunity by favoring the development of Foxp3-expressing regulatory CD4⁺ T cells. *J. Immunol.* **176**, 839–847.
- Fazilleau N., Delarasse C., Sweeney C. H., Anderton S. M., Fillatreau S., Lemonnier F. A., Pham-Dinh D. and Kanellopoulos J. M. (2006) Persistence of autoreactive myelin oligodendrocyte glycoprotein (MOG)-specific T cell repertoires in MOG-expressing mice. *Eur. J. Immunol.* **36**, 533–543.
- Fehervari Z. and Sakaguchi S. (2004) Control of Foxp3⁺ CD25⁺CD4⁺ regulatory cell activation and function by dendritic cells. *Int. Immunol.* **16**, 1769–1780.
- Feng J. M., Givogri I. M., Bongarzone E. R., Campagnoni C., Jacobs E., Handley V. W., Schonmann V. and Campagnoni A. T. (2000) Thymocytes express the golli products of the myelin basic protein

- gene and levels of expression are stage dependent. *J. Immunol.* **165**, 5443–5450.
- Fisson S., Darrasse-Jeze G., Litvinova E., Septier F., Klatzmann D., Liblau R. and Salomon B. L. (2003) Continuous activation of autoreactive CD4⁺ CD25⁺ regulatory T cells in the steady state. *J. Exp. Med.* **198**, 737–746.
- Fisson S., Djelti F., Trenado A., Billiard F., Liblau R., Klatzmann D., Cohen J. L. and Salomon B. L. (2006) Therapeutic potential of self-antigen-specific CD4⁺ CD25⁺ regulatory T cells selected *in vitro* from a polyclonal repertoire. *Eur. J. Immunol.* **36**, 817–827.
- Fontenot J. D., Rasmussen J. P., Williams L. M., Dooley J. L., Farr A. G. and Rudensky A. Y. (2005) Regulatory T cell lineage specification by the forkhead transcription factor foxp3. *Immunity* **22**, 329–341.
- Gartner D., Hoff H., Gimsa U., Burmester G. R. and Brunner-Weinzler M. C. (2006) CD25 regulatory T cells determine secondary but not primary remission in EAE: impact on long-term disease progression. *J. Neuroimmunol.* **172**, 73–84.
- Genain C. P., Cannella B., Hauser S. L. and Raine C. S. (1999) Identification of autoantibodies associated with myelin damage in multiple sclerosis. *Nat. Med.* **5**, 170–175.
- Ghiringhelli F., Menard C., Terme M. *et al.* (2005) CD4⁺CD25⁺ regulatory T cells inhibit natural killer cell functions in a transforming growth factor- β -dependent manner. *J. Exp. Med.* **202**, 1075–1085.
- Gotter J., Brors B., Hergenroth M. and Kyewski B. (2004) Medullary epithelial cells of the human thymus express a highly diverse selection of tissue-specific genes colocalized in chromosomal clusters. *J. Exp. Med.* **199**, 155–166.
- Grajewski R. S., Silver P. B., Agarwal R. K., Su S. B., Chan C. C., Liou G. I. and Caspi R. R. (2006) Endogenous IRBP can be dispensable for generation of natural CD4⁺CD25⁺ regulatory T cells that protect from IRBP-induced retinal autoimmunity. *J. Exp. Med.* **203**, 851–856.
- Greter M., Heppner F. L., Lemos M. P., Odermatt B. M., Goebels N., Laufer T., Noelle R. J. and Becher B. (2005) Dendritic cells permit immune invasion of the CNS in an animal model of multiple sclerosis. *Nat. Med.* **11**, 328–334.
- Grossman W. J., Verbsky J. W., Barchet W., Colonna M., Atkinson J. P. and Ley T. J. (2004) Human T regulatory cells can use the perforin pathway to cause autologous target cell death. *Immunity* **21**, 589–601.
- Haas J., Hug A., Viehaver A. *et al.* (2005) Reduced suppressive effect of CD4⁺CD25^{high} regulatory T cells on the T cell immune response against myelin oligodendrocyte glycoprotein in patients with multiple sclerosis. *Eur. J. Immunol.* **35**, 3343–3352.
- Hickey W. F., Hsu B. L. and Kimura H. (1991) T-lymphocyte entry into the central nervous system. *J. Neurosci. Res.* **28**, 254–260.
- Hong J., Li N., Zhang X., Zheng B. and Zhang J. Z. (2005) Induction of CD4⁺CD25⁺ regulatory T cells by co-polymer-I through activation of transcription factor Foxp3. *Proc. Natl Acad. Sci. USA* **102**, 6449–6454.
- Hori S., Haurly M., Coutinho A. and Demengeot J. (2002) Specificity requirements for selection and effector functions of CD25⁺ regulatory T cells in anti-myelin basic protein T cell receptor transgenic mice. *Proc. Natl Acad. Sci. USA* **99**, 8213–8218.
- Hsieh C. S., Liang Y., Tyznik A. J., Self S. G., Liggitt D. and Rudensky A. Y. (2004) Recognition of the peripheral self by naturally arising CD25⁺ CD4⁺ T cell receptors. *Immunity* **21**, 267–277.
- Huan J., Culbertson N., Spencer L., Bartholomew R., Burrows G. G., Chou Y. K., Bourdette D., Ziegler S. F., Offner H. and Vandenbark A. A. (2005) Decreased FOXP3 levels in multiple sclerosis patients. *J. Neurosci. Res.* **81**, 45–52.
- Huseby E. S., Sather B., Huseby P. G. and Goverman J. (2001) Age-dependent T cell tolerance and autoimmunity to myelin basic protein. *Immunity* **14**, 471–481.
- Issazadeh S., Navikas V., Schaub M., Sayegh M. and Khoury S. (1998) Kinetics of expression of co-stimulatory molecules and their ligands in murine relapsing experimental autoimmune encephalomyelitis *in vivo*. *J. Immunol.* **161**, 1104–1112.
- Jordan M. S., Boesteanu A., Reed A. J., Petrone A. L., Hohenbeck A. E., Lerman M. A., Naji A. and Caton A. J. (2001) Thymic selection of CD4⁺CD25⁺ regulatory T cells induced by an agonist self-peptide. *Nat. Immunol.* **2**, 301–306.
- Kerlero de Rosbo N., Milo R., Lees M. B., Burger D., Bernard C. C. and Ben-Nun A. (1993) Reactivity to myelin antigens in multiple sclerosis. Peripheral blood lymphocytes respond predominantly to myelin oligodendrocyte glycoprotein. *J. Clin. Invest.* **92**, 2602–2608.
- Khoury S. J., Hancock W. W. and Weiner H. L. (1992) Oral tolerance to myelin basic protein and natural recovery from experimental autoimmune encephalomyelitis are associated with downregulation of inflammatory cytokines and differential upregulation of transforming growth factor β , interleukin 4, and prostaglandin E expression in the brain. *J. Exp. Med.* **176**, 1355–1364.
- Kivisakk P., Mahad D. J., Callahan M. K. *et al.* (2004) Expression of CCR7 in multiple sclerosis: implications for CNS immunity. *Ann. Neurol.* **55**, 627–638.
- Klein L., Klugmann M., Nave K. A., Tuohy V. K. and Kyewski B. (2000) Shaping of the autoreactive T-cell repertoire by a splice variant of self protein expressed in thymic epithelial cells. *Nat. Med.* **6**, 56–61.
- Kohm A. P., Carpentier P. A., Anger H. A. and Miller S. D. (2002) Cutting edge: CD4⁺CD25⁺ regulatory T cells suppress antigen-specific autoreactive immune responses and central nervous system inflammation during active experimental autoimmune encephalomyelitis. *J. Immunol.* **169**, 4712–4716.
- Kohm A. P., Williams J. S. and Miller S. D. (2004) Cutting edge: ligation of the glucocorticoid-induced TNF receptor enhances autoreactive CD4⁺ T cell activation and experimental autoimmune encephalomyelitis. *J. Immunol.* **172**, 4686–4690.
- Kojima K., Reindl M., Lassmann H., Wekerle H. and Linington C. (1997) The thymus and self-tolerance: co-existence of encephalitogenic S100 β -specific T cells and their nominal autoantigen in the normal adult rat thymus. *Int. Immunol.* **9**, 897–904.
- Krakovski M. L. and Owens T. (2000) Naive T lymphocytes traffic to inflamed central nervous system, but require antigen recognition for activation. *Eur. J. Immunol.* **30**, 1002–1009.
- Kretschmer K., Apostolou I., Hawiger D., Khazaie K., Nussenzweig M. C. and von Boehmer H. (2005) Inducing and expanding regulatory T cell populations by foreign antigen. *Nat. Immunol.* **6**, 1219–1227.
- Kuchroo V. K., Anderson A. C., Waldner H., Munder M., Bettelli E. and Nicholson L. B. (2002) T cell response in experimental autoimmune encephalomyelitis (EAE): role of self and cross-reactive antigens in shaping, tuning, and regulating the autopathogenic T cell repertoire. *Annu. Rev. Immunol.* **20**, 101–123.
- Kyewski B. and Klein L. (2006) A central role for central tolerance. *Annu. Rev. Immunol.* **24**, 571–606.
- Langrish C. L., Chen Y., Blumenschein W. M., Mattson J., Basham B., Sedgwick J. D., McClanahan T., Kastelein R. A. and Cua D. J. (2005) IL-23 drives a pathogenic T cell population that induces autoimmune inflammation. *J. Exp. Med.* **201**, 233–240.
- Lim H. W., Hillsamer P., Banham A. H. and Kim C. H. (2005) Cutting edge: direct suppression of B cells by CD4⁺ CD25⁺ regulatory T cells. *J. Immunol.* **175**, 4180–4183.

- Linares D., Mana P., Goodyear M. *et al.* (2003) The magnitude and encephalogenic potential of autoimmune response to MOG is enhanced in MOG deficient mice. *J. Autoimmun.* **21**, 339–351.
- Liston A., Lesage S., Wilson J., Peltonen L. and Goodnow C. C. (2003) Aire regulates negative selection of organ-specific T cells. *Nat. Immunol.* **4**, 350–354.
- Liston A., Gray D. H., Lesage S., Fletcher A. L., Wilson J., Webster K. E., Scott H. S., Boyd R. L., Peltonen L. and Goodnow C. C. (2004) Gene dosage – limiting role of Aire in thymic expression, clonal deletion, and organ-specific autoimmunity. *J. Exp. Med.* **200**, 1015–1026.
- Liu H., MacKenzie-Graham A. J., Kim S. and Voskuhl R. R. (2001) Mice resistant to experimental autoimmune encephalomyelitis have increased thymic expression of myelin basic protein and increased MBP-specific T cell tolerance. *J. Neuroimmunol.* **115**, 118–126.
- Liu W., Putnam A. L., Xu-Yu Z. *et al.* (2006a) CD127 expression inversely correlates with FoxP3 and suppressive function of human CD4⁺ T reg cells. *J. Exp. Med.* **203**, 1701–1711.
- Liu Y., Teige I., Birnir B. and Issazadeh-Navikas S. (2006b) Neuron-mediated generation of regulatory T cells from encephalitogenic T cells suppresses EAE. *Nat. Med.* **12**, 518–525.
- Mannie M. D., Prevost K. D. and Marinakis C. A. (1995) Prostaglandin E2 promotes the induction of anergy during T helper cell recognition of myelin basic protein. *Cell Immunol.* **160**, 132–138.
- Masteller E. L., Warner M. R., Tang Q., Tarbell K. V., McDevitt H. and Bluestone J. A. (2005) Expansion of functional endogenous antigen-specific CD4⁺CD25⁺ regulatory T cells from non-obese diabetic mice. *J. Immunol.* **175**, 3053–3059.
- McGeachy M. J., Stephens L. A. and Anderton S. M. (2005) Natural recovery and protection from autoimmune encephalomyelitis: contribution of CD4⁺CD25⁺ regulatory cells within the central nervous system. *J. Immunol.* **175**, 3025–3032.
- McMahon E. J., Bailey S. L., Castenada C. V., Waldner H. and Miller S. D. (2005) Epitope spreading initiates in the CNS in two mouse models of multiple sclerosis. *Nat. Med.* **11**, 335–339.
- McRae B. L., Vanderlugt C. L., Dal Canto M. C. and Miller S. D. (1995) Functional evidence for epitope spreading in the relapsing pathology of experimental autoimmune encephalomyelitis. *J. Exp. Med.* **182**, 75–85.
- Mekala D. J., Alli R. S. and Geiger T. L. (2005) IL-10-dependent infectious tolerance after the treatment of experimental allergic encephalomyelitis with redirected CD4⁺CD25⁺ T lymphocytes. *Proc. Natl Acad. Sci. USA* **102**, 11 817–11 822.
- Montero E., Nussbaum G., Kaye J. F., Perez R., Lage A., Ben-Nun A. and Cohen I. R. (2004) Regulation of experimental autoimmune encephalomyelitis by CD4⁺, CD25⁺ and CD8⁺ T cells: analysis using depleting antibodies. *J. Autoimmun.* **23**, 1–7.
- Ochi H., Abraham M., Ishikawa H. *et al.* (2006) Oral CD3-specific antibody suppresses autoimmune encephalomyelitis by inducing CD4⁺ CD25⁺ LAP⁺ T cells. *Nat. Med.* **12**, 627–635.
- Olivares-Villagomez D., Wang Y. and Lafaille J. J. (1998) Regulatory CD4⁺ T cells expressing endogenous T cell receptor chains protect myelin basic protein-specific transgenic mice from spontaneous autoimmune encephalomyelitis. *J. Exp. Med.* **188**, 1883–1894.
- Olsson T., Sun J., Hillert J., Hojberg B., Ekre H. P., Andersson G., Olerup O. and Link H. (1992) Increased numbers of T cells recognizing multiple myelin basic protein epitopes in multiple sclerosis. *Eur. J. Immunol.* **22**, 1083–1087.
- Pasare C. and Medzhitov R. (2003) Toll pathway-dependent blockade of CD4⁺CD25⁺ T cell-mediated suppression by dendritic cells. *Science* **299**, 1033–1036.
- Perchellet A., Stromnes I., Pang J. M. and Goverman J. (2004) CD8⁺ T cells maintain tolerance to myelin basic protein by 'epitope theft'. *Nat. Immunol.* **5**, 606–614.
- Perry V. H. (1998) A revised view of the central nervous system microenvironment and major histocompatibility complex class II antigen presentation. *J. Neuroimmunol.* **90**, 113–121.
- Peterson P., Nagamine K., Scott H., Heino M., Kudoh J., Shimizu N., Antonarakis S. E. and Krohn K. J. (1998) APECED: a monogenic autoimmune disease providing new clues to self-tolerance. *Immunol. Today* **19**, 384–386.
- Pribyl T. M., Campagnoni C. W., Kampf K., Kashima T., Handley V. W., McMahon J. and Campagnoni A. T. (1996) Expression of the myelin proteolipid protein gene in the human fetal thymus. *J. Neuroimmunol.* **67**, 125–130.
- Pugliatti M., Sotgiu S., Solinas G., Castiglia P., Pirastru M. I., Murgia B., Mannu L., Sanna G. and Rosati G. (2001) Multiple sclerosis epidemiology in Sardinia: evidence for a true increasing risk. *Acta Neurol. Scand.* **103**, 20–26.
- Reddy J., Illes Z., Zhang X., Encinas J., Pyrdol J., Nicholson L., Sobel R. A., Wucherpfennig K. W. and Kuchroo V. K. (2004) Myelin proteolipid protein-specific CD4⁺CD25⁺ regulatory cells mediate genetic resistance to experimental autoimmune encephalomyelitis. *Proc. Natl Acad. Sci. USA* **101**, 15 434–15 439.
- Romagnoli P., Hudrisier D. and van Meerwijk J. P. (2002) Preferential recognition of self antigens despite normal thymic deletion of CD4⁺ CD25⁺ regulatory T cells. *J. Immunol.* **168**, 1644–1648.
- Sakaguchi S. (2004) Naturally arising CD4⁺ regulatory T cells for immunologic self-tolerance and negative control of immune responses. *Annu. Rev. Immunol.* **22**, 531–562.
- Sakaguchi S., Ono M., Setoguchi R., Yagi H., Hori S., Fehervari Z., Shimizu J., Takahashi T. and Nomura T. (2006) Foxp3CD25CD4 natural regulatory T cells in dominant self-tolerance and autoimmune disease. *Immunol. Rev.* **212**, 8–27.
- van Santen H. M., Benoist C. and Mathis D. (2004) Number of T reg cells that differentiate does not increase upon encounter of agonist ligand on thymic epithelial cells. *J. Exp. Med.* **200**, 1221–1230.
- Seddiki N., Santner-Nanan B., Martinson J. *et al.* (2006) Expression of interleukin (IL)-2 and IL-7 receptors discriminates between human regulatory and activated T cells. *J. Exp. Med.* **203**, 1693–1700.
- Siggs O. M., Makaroff L. E. and Liston A. (2006) The why and how of thymocyte negative selection. *Curr. Opin. Immunol.* **18**, 175–183.
- Sospedra M. and Martin R. (2005) Immunology of multiple sclerosis. *Annu. Rev. Immunol.* **23**, 683–747.
- Stephens L. A., Gray D. and Anderton S. M. (2005) CD4⁺CD25⁺ regulatory T cells limit the risk of autoimmune disease arising from T cell receptor crossreactivity. *Proc. Natl Acad. Sci. USA* **102**, 17 418–17 423.
- Stern J. N., Illes Z., Reddy J. *et al.* (2004) Amelioration of proteolipid protein 139–151-induced encephalomyelitis in SJL mice by modified amino acid copolymers and their mechanisms. *Proc. Natl Acad. Sci. USA* **101**, 11 743–11 748.
- van Stipdonk M. J., Willems A. A., Plomp A. C., van Noort J. M. and Boog C. J. (2000) Tolerance controls encephalitogenicity of α B-crystallin in the Lewis rat. *J. Neuroimmunol.* **103**, 103–111.
- Suvannavejh G. C., Dal Canto M. C., Matis L. A. and Miller S. D. (2000) Fas-mediated apoptosis in clinical remissions of relapsing experimental autoimmune encephalomyelitis. *J. Clin. Invest.* **105**, 223–231.
- Tang Q., Henriksen K. J., Bi M., Finger E. B., Szot G., Ye J., Masteller E. L., McDevitt H., Bonyhadi M. and Bluestone J. A. (2004) *In vitro*-expanded antigen-specific regulatory T cells suppress autoimmune diabetes. *J. Exp. Med.* **199**, 1455–1465.
- Tarbell K. V., Yamazaki S., Olson K., Toy P. and Steinman R. M. (2004) CD25⁺ CD4⁺ T cells, expanded with dendritic cells presenting a single autoantigenic peptide, suppress autoimmune diabetes. *J. Exp. Med.* **199**, 1467–1477.

- Thornton A. M., Piccirillo C. A. and Shevach E. M. (2004) Activation requirements for the induction of CD4+CD25+ T cell suppressor function. *Eur. J. Immunol.* **34**, 366–376.
- Tischner D., Weishaupt A., van den Brandt J. *et al.* (2006) Polyclonal expansion of regulatory T cells interferes with effector cell migration in a model of multiple sclerosis. *Brain* **129**, 2635–2647.
- Tsaknaris L., Spencer L., Culbertson N. *et al.* (2003) Functional assay for human CD4+CD25+ Treg cells reveals an age-dependent loss of suppressive activity. *J. Neurosci. Res.* **74**, 296–308.
- Tuohy V. K., Yu M., Yin L., Kawczak J. A. and Kinkel R. P. (1999) Spontaneous regression of primary autoreactivity during chronic progression of experimental autoimmune encephalomyelitis and multiple sclerosis. *J. Exp. Med.* **189**, 1033–1042.
- Venken K., Hellings N., Hensen K., Rummens J. L., Medaer R., D'Hooghe M. B., Dubois B., Raus J. and Stinissen P. (2006) Secondary progressive in contrast to relapsing-remitting multiple sclerosis patients show a normal CD4+CD25+ regulatory T-cell function and FOXP3 expression. *J. Neurosci. Res.* **83**, 1432–1446.
- Viglietta V., Baecher-Allan C., Weiner H. L. and Hafler D. A. (2004) Loss of functional suppression by CD4+CD25+ regulatory T cells in patients with multiple sclerosis. *J. Exp. Med.* **199**, 971–979.
- Waldner H., Collins M. and Kuchroo V. K. (2004) Activation of antigen-presenting cells by microbial products breaks self tolerance and induces autoimmune disease. *J. Clin. Invest.* **113**, 990–997.
- Walker M. R., Carson B. D., Nepom G. T., Ziegler S. F. and Buckner J. H. (2005) De novo generation of antigen-specific CD4+CD25+ regulatory T cells from human CD4+CD25- cells. *Proc. Natl Acad. Sci. USA* **102**, 4103–4108.
- Walker L. S., Chodos A., Eggena M., Dooms H. and Abbas A. K. (2003) Antigen-dependent proliferation of CD4+ CD25+ regulatory T cells *in vivo*. *J. Exp. Med.* **198**, 249–258.
- Weber S. E., Harbertson J., Godebu E., Mros G. A., Padrick R. C., Carson B. D., Ziegler S. F. and Bradley L. M. (2006) Adaptive islet-specific regulatory CD4 T cells control autoimmune diabetes and mediate the disappearance of pathogenic Th1 cells *in vivo*. *J. Immunol.* **176**, 4730–4739.
- Yamazaki S., Iyoda T., Tarbell K., Olson K., Velinzon K., Inaba K. and Steinman R. M. (2003) Direct expansion of functional CD25+ CD4+ regulatory T cells by antigen-processing dendritic cells. *J. Exp. Med.* **198**, 235–247.
- Yu P., Gregg R. K., Bell J. J. *et al.* (2005) Specific T regulatory cells display broad suppressive functions against experimental allergic encephalomyelitis upon activation with cognate antigen. *J. Immunol.* **174**, 6772–6780.
- Zamvil S. S. and Steinman L. (2003) Diverse targets for intervention during inflammatory and neurodegenerative phases of multiple sclerosis. *Neuron* **38**, 685–688.
- Zhang X., Koldzic D. N., Izikson L., Reddy J., Nazareno R. F., Sakaguchi S., Kuchroo V. K. and Weiner H. L. (2004) IL-10 is involved in the suppression of experimental autoimmune encephalomyelitis by CD25+CD4+ regulatory T cells. *Int. Immunol.* **16**, 249–256.
- Zhang X., Reddy J., Ochi H., Frenkel D., Kuchroo V. K. and Weiner H. L. (2006) Recovery from experimental allergic encephalomyelitis is TGF- β dependent and associated with increases in CD4+LAP+ and CD4+CD25+ T cells. *Int. Immunol.* **18**, 495–503.
- Zhu C., Anderson A. C., Schubart A., Xiong H., Imitola J., Khoury S. J., Zheng X. X., Strom T. B. and Kuchroo V. K. (2005) The Tim-3 ligand galectin-9 negatively regulates T helper type 1 immunity. *Nat. Immunol.* **6**, 1245–1252.
- Ziegler S. F. (2006) FOXP3: of mice and men. *Annu. Rev. Immunol.* **24**, 209–226.