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## MINI-REVIEW

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

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<sup>†</sup>Immunology Laboratory Toulouse University Hospital, Toulouse, France<sup>‡</sup>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<sup>+</sup>Foxp3<sup>+</sup> 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.

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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<sup>+</sup> and CD8<sup>+</sup> 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

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*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<sup>+</sup>Foxp3<sup>+</sup> 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 E<sub>2</sub>, TGF- $\beta$ , 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<sup>+</sup> and CD8<sup>+</sup> 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).

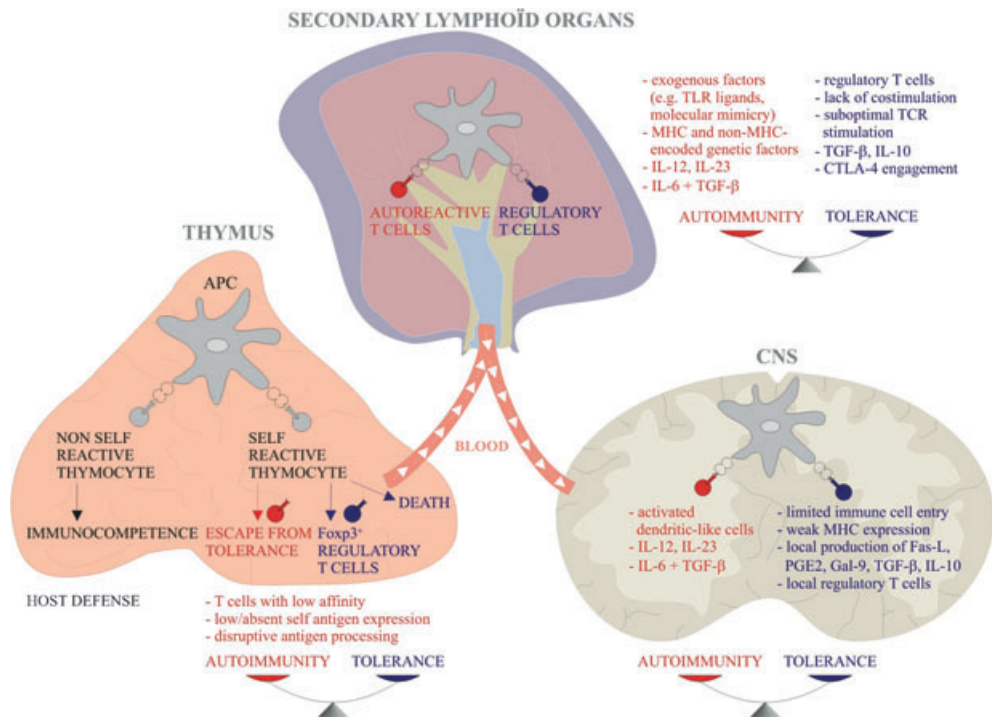
Second, antigen presentation does occur in the CNS. Indeed, upon exposure to a pro-inflammatory environment, oligodendrocytes and neurons express MHC class I molecules (allowing antigen presentation to CD8<sup>+</sup> T cells), while astrocytes and microglial cells can express both MHC class I and II molecules (allowing antigen presentation to both CD8<sup>+</sup> and CD4<sup>+</sup> T cells). Moreover, it has been shown that dendritic-like cells are necessary and sufficient to reactivate CD4<sup>+</sup> 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- $\beta$  long known to exhibit regulatory properties is now being incriminated in pathogenic processes. Indeed, contingent upon an inflammatory cytokine environment, TGF- $\beta$  also promotes the differentiation of CD4<sup>+</sup> 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<sup>+/+</sup> versus MBP<sup>-/-</sup> 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<sup>+</sup> T cells are primarily specific for PLP<sub>139–151</sub>, 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<sub>139–151</sub> 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<sup>+/+</sup> versus MOG<sup>-/-</sup> mouse (Linares *et al.* 2003). However, independent data comparing MOG-specific T cell responses in MOG<sup>-/-</sup> vs. MOG<sup>+/+</sup> 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 $\beta$ , 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 $\beta$ -specific CD4<sup>+</sup> 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  $\alpha$ 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  $\alpha$ 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<sup>+</sup>CD25<sup>+</sup>Foxp3<sup>+</sup> phenotype are the most studied and best characterized (Sakaguchi *et al.* 2006). CD4<sup>+</sup>CD25<sup>+</sup>Foxp3<sup>+</sup> 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<sup>+</sup> 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<sup>+</sup>CD25<sup>+</sup> 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<sup>+</sup> and CD8<sup>+</sup> 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<sup>+</sup> T cells and 3–9% of human circulating CD4<sup>+</sup> T cells (Seddiki *et al.* 2006). They control the activation of conventional CD4<sup>+</sup> T cells, but also CD8<sup>+</sup> 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<sup>-/-</sup> MBP-TCR transgenic mice, transfer of CD4<sup>+</sup> or CD4<sup>+</sup>CD25<sup>+</sup> 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<sup>+</sup>CD25<sup>+</sup> 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<sup>+</sup>CD25<sup>+</sup> 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<sup>+</sup>CD25<sup>high</sup> 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<sup>+/+</sup> but not in IRBP<sup>-/-</sup> 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<sub>139–151</sub>, 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<sup>+</sup>CD25<sup>+</sup>Foxp3<sup>+</sup> 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<sup>+</sup> 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<sup>+</sup> 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<sup>+</sup> 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- $\beta$  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<sup>+</sup> T cells into Tregs by forced expression of the transcription factor Foxp3. Mouse CD4<sup>+</sup> 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<sub>139–151</sub>-specific CD4<sup>+</sup> cells still exhibit encephalitogenic properties after transfer into Rag<sup>-/-</sup> 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<sup>+</sup> 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<sup>+</sup> 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<sup>+</sup> 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<sub>139–151</sub>-specific Tregs are able to control PLP<sub>139–151</sub>-induced EAE but not MBP<sub>87–99</sub>-induced EAE (Yu *et al.* 2005). However, when pre-activated *in vitro* prior to injection, PLP<sub>139–151</sub>-specific Tregs suppress both PLP<sub>139–151</sub> and MBP<sub>87–99</sub>-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.

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