The role of selected immunoregulatory cell populations in autoimmune demyelination

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Abstract

Much experimental and clinical evidence has been accumulated indicating the complexity of regulatory processes associated with autoimmune demyelination. Even slight disbalance of immunoregulatory circuits may result in the loss of proper control of self antigen specific immune reaction. Here, we discuss the immunoregulatory potential of several immune (dendritic cells and regulatory T cells), as well as non-immune cell populations (mesenchymal stem cells and astrocytes) with regard to their possible role in autoimmune demyelination.

REVIEW

Abbreviations:

CNS	- central nervous system
MS	- multiple sclerosis
EAE	- experimental autoimmune encephalomyelitis
APC	- antigen-presenting cell
DCs	- dendritic cells
Tregs	- regulatory T cells
nTregs	- natural Tregs
iTregs	- inducible Tregs
SC	- stem cells
PBMCs	- peripheral blood mononuclear cells
TLR	- Toll-like receptor
iNOS	- inducible nitric oxide synthase
IDO	- indoleamine-2,3-dioxygenase
IFN	- interferon
IL	- interleukin

INTRODUCTION

The results of numerous studies point at the autoaggressive mechanisms underlying the demyelination of the central nervous system (CNS) characteristic for multiple sclerosis (MS) (Probert & Selmaj 1997; Hohlfeld *et al.* 1995; Hafler & Weiner 1995). The morphological sign of the ongoing pathological process is the focal damage of the myelin sheath associated with the inflammatory infiltration – so called "demyelination plaque" (Raine 1997). Several types of CNS lesions were described showing diverse signs of accumulation of cellular and humoral components of the immune system, as well as different demyelination and remyelination patterns (Lucchinetti *et al.* 2000). Also particular types of the clinical course of the disease, which may be relapsing-remitting, secondary progressive or primary progressive, are accompanied by distinctive immunopathological changes (Bramow *et al.* 2010). Apart from demy-

elination, an axonal loss was documented in MS lesions and axonal injury was suggested as the main correlate of the functional disability in MS patients (Bjartmar *et al.* 2000). In the last years, the correlation between the immune processes and axonal and neuronal degeneration in MS became a topic of a serious scientific discussion (DeLuca *et al.* 2006). However, the latest studies seem to confirm the primary role of the inflammatory reaction in the pathological cascade underlying the CNS changes in MS (Frischer *et al.* 2009; Gunnarsson *et al.* 2010).

On the cellular level, the immunopathological observations suggest the oligodendrocyte as a target for the autoimmune response in MS and myelin components as potential antigens provoking the uncontrolled development of immune reaction (Raine *et al.* 1997). Some of these proteins (mainly MBP, PLP, MOG) have successfully been used in rodents to induce, after subcutaneous immunization, the experimental autoimmune encephalomyelitis (EAE). The disease representing many pathological and clinical features of MS is considered as the best available animal model of MS (Hafler & Weiner 1995; Owens *et al.* 2001). The experimental results suggest that myelin specific T cell clones are the effector component of the autoaggressive process in EAE (Kuchroo *et al.* 2002). According to recently published observations, the cooperative action of lymphocytes secreting interferon (IFN)–γ (T helper type 1; Th1 cells) and interleukin (IL)-17 (Th17 cells) seems to be crucial for EAE development (Gocke *et al.* 2007; O'Connor *et al.* 2008). The association of disease activity and therapy response with accumulation of Th1 and Th17 cells and enhanced IFN-γ and IL-17 production was also shown in MS (Frisullo *et al.* 2008; Kebir *et al.* 2009; Axtell *et al.* 2010), supporting the putative engagement of specific T cell clones in the effector phase of the autoaggressive demyelination in humans. However, nearly two decades ago it has been demonstrated that myelin specific autoreactive T cells are a "normal" part of the immune repertoire also in healthy individuals (Martin *et al.* 1992). This fact implies the existence of immunoregulatory abnormalities in MS which eventually lead to uncontrolled activation of autoreactive effector cells, demyelination, inefficient remyelination, axonal loss and clinical deficits.

Immunoregulation in humans and other mammals encompasses very complex systemic and local processes, depending on the activity of numerous regulatory cell types, as well as associated with them transcriptional factors, enzymes and broad array of cell-surface and humoral molecules. The mechanisms preventing, under healthy conditions, autoaggressive reactions include not only immune cell populations involved in central and peripheral tolerance – mainly dendritic cells (DCs) and regulatory T cells, but also cell types from outside the classical immune system, e.g. stem cells and organ specific cells taking part in the local immunoregulatory processes, e.g. astrocytes in CNS.

DENDRITIC CELLS

Expression of a broad spectrum of pattern recognition receptors (e.g., Toll Like Receptors, TLRs) enables DCs to react directly to various pathogen-specific elements and – as a result – affect the direction and intensity of the innate immune response (Banchereau & Steinman 1998; Shortman & Liu 2002). Additionally, as the most effective antigen presenting cells (APC), DCs are crucial regulators of the adoptive immunity, linking in that way both major arms of the host defence. DCs prime the differentiation of naive T and B cells (Adema *et al.* 1997; Croft *et al.* 1992). Also, they are able to present the exogenous antigens bound to both MHC class II and MHC class I molecules. This process known as a "cross presentation" facilitates immune reaction against viral or tumour antigens obtained from dead cells ("cross activation") and enables induction and maintaining of peripheral tolerance towards self-antigens ("cross tolerance") (Brossart & Bevan 1997). In the thymus, DCs take part in central tolerance by detecting and deleting self-reactive thymocytes in the process of negative selection (Volkmann *et al.* 1997). However, the functional properties depend strongly on the subset and maturation state of DCs. In humans, there are two main DC-subsets – myeloid and plasmacytoid DCs (mDCs and pDCs, respectively). These subsets differ in phenotype and function including: ability to capture, process and present antigens; secretion profile; reactivity to microbial products and the type of immune reaction preferentially primed. The myeloid subset typically secretes large amounts of IL-12 and IL-23 upon stimulation and, thus, is thought to be responsible for induction and propagation of Th1- and Th17-driven immune responses (Oppmann *et al.* 2000; Aggrawal *et al.* 2010). To the contrary, pDCs are characterized by the ability to secrete very high amounts of type I IFNs (mainly IFN-α and IFN-β) and produce IL-12 only under particular experimental conditions. Concomitantly with other secretion profile, pDCs are not only able to prime Th1 cells (Cella *et al.* 2000) but rather demonstrate a bias to induce differentiation of Th2 cells (Rissoan *et al.* 1999), as well as regulatory T cells (Moseman *et al.* 2004; Ito *et al.* 2007). Also the pattern of Toll-like receptor (TLR) expression on pDCs, consisting mainly of TLR7 and TLR9 and very low level of other TLRs, differs strikingly from other DC subsets (Kadowaki *et al.* 2001).

Numerous EAE studies proved the engagement of DCs in the regulation of the immune processes leading to the autoimmune demyelination in this animal model. DCs were not only very efficient stimulators of encephalitogenic T cells, but were also able, after previous incubation with immunogenic peptide, to transfer disease to healthy animals (Dittel *et al.* 1999; Weir *et al.* 2002). In the natural course of EAE, the accumulation and maturity state of DCs in CNS correlated with clinical signs of the disease (Serafini *et al.* 2000; Fischer & Reichmann 2001). On the other hand, depending on the

state of maturation and the way of administration, DCs presenting myelin antigens could influence beneficially the clinical course of EAE (Huang *et al.* 2000; Menges *et al.* 2002). Additionally, it was demonstrated that diverse DCs subsets may exert different or even contrary effects on CNS immune reaction, with pDCs acting as suppressors of mDC-stimulated TH17 response (Bailey *et al.* 2007; Bailey-Bucktrout *et al.* 2008).

In contrast to EAE, data regarding the involvement of DCs in immunopathogenesis of MS are rather sparse and – to great extent – address the properties of DCs derived from human monocytes *in vitro* (Huang *et al.* 1999; Huang *et al.* 2001a; Huang *et al.* 2001b; Hussien *et al.* 2001; Berghella *et al.*, 2005). However, both myeloid and plasmacytoid DCs were found in cerebrospinal fluid of MS patients (Pashenkov *et al.* 2001). In our research, we performed the phenotypic and functional analysis of the three main peripheral blood DCs populations (two distinct populations of mDCs and pDC) in patients with relapsing-remitting MS and in healthy subjects. There were no differences in the frequency of the particular DC populations between MS patients and healthy subjects. We found, however, that peripheral blood pDCs in MS patients showed significantly reduced expression of two main co-stimulatory molecules: CD86 and 4-1BBL, while both myeloid DCs populations did not differ phenotypically between MS and healthy subjects. The immature co-stimulatory molecule profile of freshly isolated pDCs in MS was further confirmed in culture experiments. Plasmacytoid DCs, isolated from MS patients and cultured under conditions mimicking to some extent maturation signals associated with acquired immune reaction (IL-3 and CD40L), showed impaired up-regulation of several molecules crucial in the DC–effector cell interaction (CD40, CD83, CD86, 4-1BBL). In further experiments we demonstrated also that pDCs from MS patients failed to regulate the proliferative and secretory response of autologous peripheral blood mononuclear cells (PBMCs) (Stasiolek *et al.* 2006).

In order to assess the functional properties of pDCs with regard to their engagement in innate immunity, we stimulated pDCs with TLR7 and TLR9 ligands. Under these conditions, which imitate an influence of microbial products, pDCs from MS patients were able to overcome the observed *ex vivo* phenotypic deficits, however, secreted significantly lower amounts of IFN-α than pDCs from healthy subjects. Additionally, the pDCs expression profile of other TLR receptors was significantly different in MS than in controls (Stasiolek *et al.* 2006; Bayas *et al.* 2009). Recently, the pDCs population was further divided into two functionally different subsets. In relapsing-remitting MS patients the balance between these two subsets was demonstrated to be disturbed, resulting in a proinflammatory bias of the pDC-primed immune response (Schwab *et al.* 2010). Also in progressive forms of MS, the peripheral blood DCs were shown to posses an immature surface expression profile (Lopez *et al.* 2006). Most recently, an accumulation of pDCs in cerebrospinal fluid was observed in MS patients suffering from the acute disease relapse, supporting the experimental data which demonstrate an engagement of pDCs in the local CNS immunity (Longhini *et al.* 2011).

Interestingly, we observed that the phenotypic and functional abnormalities of pDCs in MS could be reversed, at least partially, by immunomodulatory treatment with glatiramer acetate (Copaxone) (Stasiolek *et al.* 2006). The influence of the MS therapy on the DCs properties was documented also for IFN-β (Lande *et al.* 2008) and corticosteroids (Navarro *et al.* 2006).

REGULATORY T CELLS

Immunoregulatory T cells encompass a growing group of various T lymphocyte populations including: $CD4^+CD25^+FoxP3^+$T cells (Tregs) (reviewed in Curotto de Lafaille & Lafaille 2009), IL-10 secreting type 1 regulatory T cells (Tr1) (Levings & Roncarolo 2000), TGF-β producing Th3 cells (Chen *et al.* 1994) and regulatory $CD8^+$ T cells (Sun *et al.* 1988). In our research, we focused on Tregs and their involvement in MS pathology. The selection of antigen specific Tregs in thymus (natural Tregs, nTregs) has been well established in numerous studies. Moreover, accumulating evidence suggests that Tregs generated in the periphery upon encounter of foreign and self antigens (adaptive or inducible Tregs, iTregs) play an indispensable role in the maintenance of immune homeostasis (Curotto de Lafaille & Lafaille , 2009; Sakaguchi *et al.* 2006). Importantly, both natural and inducible Treg populations could be effectively expanded in experimental settings by antigen presenting DCs (Yamazaki *et al.* 2003; Yamazaki *et al.* 2007).

The antigen specific, beneficial effect of Tregs on the immune demyelination was demonstrated in various EAE models (Yu *et al.* 2005; Stephens *et al.* 2009). Additionally, regulation of Treg function and accumulation was suggested as a mechanism of action of a vast verity of EAE modulating factors including growth factors, immunoglobulins and endocrine active substances (Polanczyk *et al.* 2005; Chen *et al.* 2006; Ephrem *et al.* 2008; Platten *et al.* 2009; Benkhoucha *et al.* 2010). To the contrary, the involvement of Tregs in the immunopathology of MS is not so well understood. The frequency of Tregs in peripheral blood of relapsing-remitting MS patients was reported as reduced (Hong *et al.* 2005; Venken *et al.* 2008a; Frisullo *et al.* 2009) or equal to healthy subjects (Venken *et al.* 2006; Haas *et al.* 2005; Stasiolek *et al.* 2006; Feger *et al.* 2007). Interestingly, in some studies the results of quantitative or functional Tregs analysis were dependent on the phenotypic criteria applied (Fransson *et al.* 2009; Fletcher *et al.* 2009; Michel *et al.* 2008), underscoring the necessity of more specific phenotypic characterization of human Tregs. Nonetheless, the functional experiments, point almost

unanimously at the impairment of the regulatory properties of Tregs in MS (Vigilietta *et al.* 2004; Haas *et al.* 2005; Kumar *et al.* 2006; Frisullo *et al.* 2008; Fletcher *et al.* 2009). Interestingly, the Treg functional characteristics seems to differ between relapsing-remitting and progressive MS (Venken *et al.* 2006; Venken *et al.* 2008a; Venken *et al.* 2008b). In our study, we found similar frequencies of peripheral blood $CD4^+CD25^+FoxP3^+$ Tregs *ex vivo* in clinically stable relapsing-remitting MS patients and healthy individuals (Stasiolek *et al.* 2006). Also, the generation of Tregs from naive $CD4^+$ T cells, cultured with autologous pDCs stimulated with TLR9 ligands, did not differ between MS patients and healthy subjects (Bayas *et al.* 2009). Our further experiments demonstrated that in healthy individuals the persistence of Tregs in culture with myelin antigens depended on the presence of pDCs. To the contrary, in MS patients we observed a loss of this interaction as a sign of an impaired interplay of these two main populations of immunoregulatory cells (Stasiolek *et al.* 2006). Moreover, several studies performed with MS patients undergoing therapy showed that the same immunomodulatory agents influence and modify both DC and Treg homeostasis (Venken *et al.* 2008; Venken *et al.* 2008b; Korporal *et al.* 2008; Hong *et al.* 2005). Taken together, these observations imply the interaction between DC and Treg as a specific target for therapy development in MS.

MESENCHYMAL STEM CELLS

Mesenchymal stem cell (SC) form a cell population consisting of heterogenous stromal precursor cells with complex phenotypic and functional characteristics including ability to differentiate to various mesenchymal tissues (Pittenger *et al.* 1999; Dominici *et al.* 2006). Apart from their capacity to support tissue repair, mesenchymal SC demonstrate pronounced immunoregulatory properties. In experimental settings, mesenchymal SC have been shown to modulate a vast range of functional aspects of various immune cell types including: differentiation, proliferation and/or activation of Th1, Th2 and Th17 cells (Darlington *et al.* 2010; Ghannam *et al.* 2010; Patel *et al.* 2010; Ge *et al.* 2010), γδ T cells, natural killer (NK) cells and NK T cells (Spaggiari *et al.* 2008; Prigione *et al.* 2009); generation of Tregs (Ghannam *et al.* 2010; Patel *et al.* 2010; Ge *et al.* 2010); differentiation and immunoglobulin secretion by B cells (Asari *et al.* 2009); activation of monocytes (Cutler *et al.*, 2010); maturation and function of DCs (Spaggiari *et al.* 2009; Aldinucci *et al.* 2010); recruitment and activation of neutrophils (Brandau *et al.* 2010), as well as microglial response to microbial products (Ooi *et al.* 2010). The immunoregulatory activity of mesenchymal SC was demonstrated to be dependent both on a direct cell-to-cell contact (Aldinucci *et al.* 2010) and a variety of humoral factors including TGF-β (Patel *et al.*, 2010; Nemeth *et al.*, 2010), IL-10 (Crop *et al.* 2009), PGE_2 (Ghannam *et al.* 2010; Spaggiari *et al.* 2008), as well as soluble products of enzymatic activity of inducible nitric oxide synthase (iNOS) (Ren *et al.* 2008) and indoleamine-2,3-dioxygenase (IDO) (Spaggiari *et al.* 2008; Ge at al. 2010; Crop *et al.* 2009). The immune function of mesenchymal SC was reported as beneficial, e.g. in transplantation research (Crop *et al.* 2009; Ge *et al.* 2010) or neuroprotection (Kim *et al.* 2009; Kemp *et al.* 2010) but also as detrimental in regard to host defense against tumor cells (Patel *et al.* 2010).

The immunoregulatory role of mesenchymal SC has also been clearly demonstrated in immune demyelination. EAE studies performed in the last few years proved that intravenous (Zappia *et al.* 2005), intraperitoneal (Gordon *et al.* 2010) or intraventricular (Kassis *et al.* 2008) transfer of syngeneic (Zappia *et al.*, 2005), allogeneic (Rafei *et al.* 2009a) or even xenogeneic (Zhang *et al.* 2005) mesenchymal SC resulted in a disease protection or amelioration depending on the time-point of transplantation. The clinical effects of mesenchymal SC transplantation were on the histopathological level paralleled by reduced extent of demyelination (Zappia *et al.* 2005; Zhang *et al.* 2005; Gordon *et al.* 2010), increase in remyelination (Constantin *et al.* 2009), as well as by significant protection of axons (Constantin *et al.* 2009; Gerdoni *et al.* 2007; Zhang *et al.* 2006). In the majority of the studies, immune mechanisms associated with mesenchymal SC transfer involved suppression of antigen specific proliferation of effector cells (Zappia *et al.* 2005; Zhang *et al.* 2005; Kassis *et al.* 2008) and shift of the proinflammatory Th1/Th17 immune reaction towards Th2 response, accompanied by down-modulation of proinflammatory cytokines production (Zappia *et al.* 2005; Rafei *et al.* 2009a; Constantin *et al.* 2009; Gerdoni *et al.* 2007; Bai *et al.* 2009; Rafei 2009b). Additionally, the involvement of various growth and trophic factors has been suggested (Zhang *et al.* 2005; Zhang *et al.* 2006; Constantin *et al.* 2009; Berhum *et al.* 2010). Interestingly, there is no consensus regarding the CNS migration of transplanted SC. While some of the authors suggested peripheral lymphoid organs as a main place of immunoregulatory action of transplanted mesenchymal SC (Zappia *et al.* 2005; Gerdoni *et al.* 2007; Matysiak *et al.* 2008), others describe also clear accumulation of these cells in the demyelinated areas of CNS (Gordon *et al.* 2010, Constantin *et al.* 2009; Bai *et al.* 2009; Kassis *et al.* 2008). In our EAE experiments, we transferred intravenously bone marrow Lin^-Sca1^+ SC (a pluripotent fraction of bone marrow SC depleted of hematopoietic precursors and enriched in mesenchymal SC) to EAE animals at the peak of disease (Matysiak *et al.* 2008). The SC transplantation accelerated clinical recovery and prevented subsequent disease relapses. The clinical effect was associated with significant decrease of Wallerian degeneration, pronounced signs of diffuse remyelination and only moderate reduction of inflammation and demyelination. The transplanted SC accumulated in peripheral organs and – to a much lower extent – in

CNS, where their presence was constricted predominantly to meningeal regions. In functional experiments we demonstrated that SC transplantation resulted in an inhibition of antigen specific proliferation and abrogation of antigen spreading process, associated with high secretion of IFN-γ. Furthermore, we showed that the suppressed proliferative responses were dependent on increased IDO expression specifically in CD11+ DCs (Matysiak *et al.* 2008). These observations contribute to the complexity of immune mechanisms associated with mesenchymal SC and accentuate the role of other immunoregulatory cell types as mediators of SC activity in the periphery.

The clinical experience with the intravenous or intrathecal transplantation of mesenchymal SC in MS patients encompasses few studies with a very limited cumulative number of patients. Although we still need more data from well controlled clinical trials, the feasibility and safety of the procedure, as well as the preliminary clinical effects seem to be promising (Liang *et al.* 2009; Karussis *et al.* 2010).

ASTROCYTES

Although astrocytes are the main cellular components of the typical MS lesion (Smith & Sommer 1992), their involvement in immunopathogenesis of MS has been investigated less comprehensively than the role of immune cells or oligodendrocytes. Nonetheless, accumulating evidence suggests that astrocytes play a substantial role in the modulation of immune processes associated with autoaggressive demyelination. Expression of adhesion molecules (Archambault *et al.* 2006), metalloproteinases and their inhibitors (Teesalu *et al.* 2001; Thorne *et al.* 2009), as well as secretion of various chemokines (Calderon *et al.* 2006; Quinones *et al.* 2008) allow astrocytes to regulate trafficking of immune cells, including DCs (Ambrosini *et al.*2005), across brain-blood barrier and in CNS parenchyma. Moreover, it was demonstrated that, under inflammatory stimulation, astrocytes were able to express MHC class II and co-stimulatory molecules (Cornet el al. 2000), present myelin antigens to effector cells (Tan *et al.* 1998) and regulate the myelin specific autoimmune response (Xiao *et al.* 1998). The APC-like surface expression profile of astrocytes was also reported in active MS lesions (Ransohoff & Estes 1991; Lee *et al.* 1990). Additionally, astrocytes were shown to influence the CNS immune homeostasis by a direct cell-to-cell contact (Kim *et al.* 2010) and by surface expression or secretion of different immune active molecules, including members of TNF superfamily and nitric oxide (NO) (Thangarajh *et al.* 2007; Plant *et al.* 2005; Wilms *et al.* 2010; Raine *et al.* 1998). The production of NO by astrocytes is activated by tissue damage or inflammatory signals and depends mainly on the enzymatic activity of iNOS. Expression of iNOS have been reported in astrocytes accumulating in active MS lesions (Oleszek *et al.* 1998; Liu *et al.* 2001; Broholm *et al.* 2004) and activity of this enzyme was associated with demyelination and axonal loss (Hill *et al.* 2004; Garthwaite *et al.* 2002; Jack *et al.* 2007). The expression of iNOS, similar to the expression of various pro-inflammatory cytokines, requires activation of transcriptional factor NF-κB. In our experiments we analyzed the possibility to modulate the NF-κB signaling and iNOS expression in astroglial cells with a specific proteasome inhibitor - lactacystin (Stasiolek *et al.* 2000). Unexpectedly, we found a biphasic – inhibitory and stimulatory effect of lactacystin on the NO production induced by microbial products and pro-inflammatory cytokines. The results depended on the lactacystin concentration and the time of incubation. Moreover, delaying addition of lactacystin until several hours after inflammatory stimuli reversed the effect on iNOS activity. The differences in NO production were paralleled by modulation of iNOS expression, dependent on NF-κB activation. Interestingly, we demonstrated that the observed biphasic effects of lactacystin on iNOS promoter activity were associated with preferential increase of one of the NF-κB inhibitors, IκB-β (Stasiolek *et al.* 2000). These results, demonstrating very complex regulatory pathways in astroglial cells, are of particular meaning with regard to the possible therapeutic applications in autoimmune demyelination.

CONCLUSION

The immunoregulatory processes associated with myelin antigens are the putative place of dysfunction resulting in autoimmune demyelination and, thus, also the possible aim of new therapeutic attempts. Although our knowledge about particular cellular and humoral components of immunoregulatory circuits is constantly growing, we need to concentrate more specifically on their mutual interactions, both in the periphery and under specific conditions of CNS.

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