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Yin–Yang regulation of autoimmunity by DCs

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The key function of the immune system is to promptly signal danger by invading pathogens, while ignoring self-antigens, in which the immune cells are bathed. Autoreactive T cells, which have escaped thymic selection, are present in the normal repertoire and can be activated relatively easily and many pathogens contain lookalike determinants (mimicry epitopes) of self-antigens. To prevent induction of autoimmunity, the immune system uses a highly effective control mechanism, which efficiently discriminates between self and non-self. Peripheral tolerance to self-antigens is maintained by immature dendritic cells (iDCs), whereas mature DCs can activate autoreactive T cells. A homeostatic control mechanism operating through the balance between C-type lectin receptors (CLRs) and Toll-like receptors (TLRs) on DCs will be discussed. DC maturation induced by TLR binding of pathogen-associated molecular patterns (PAMPs) can be antagonized by CLR stimulation by specific carbohydrate structures on self-antigens and pathogens. We hypothesize that disturbed glycosylation of self-antigens might set the stage for autoimmunity by weakening the capacity of CLRs to buffer 'danger signals' through TLRs.

It is still poorly understood how the autoreactive T cells in the normal human repertoire remain in a tolerant state, even though they continuously encounter self-antigens, and why these cells are persistently activated in patients with a chronic autoimmune disease. On the basis of our research, which uses autoimmune disease models in non-human primates (the closest relative in nature to humans), we propose a regulatory mechanism exerted by dendritic cells (DCs).

Experimental autoimmune encephalomyelitis (EAE) is a prototypical animal model of organ-specific autoimmunity, in which many fundamental immunological concepts have been tested. Th1 T-cell lines specific for antigens from central nervous system (CNS) myelin have been isolated from the normal repertoire of healthy humans, as well as a variety of rodents and non-human primates. The fact that Th1 cells isolated from blood mononuclear cells of healthy monkeys induce CNS white matter inflammation when injected into compatible naïve recipients [1,2] indicates that this might also be the case in humans.

In this Opinion, we discuss how the balance of C-type lectin receptor (CLR)- and Toll-like receptor (TLR)-mediated activation signals to DCs determines whether they remain immature, and induce tolerance, or mature, and acquire the capacity to induce autoimmunity. The discussed mechanism is probably not confined to the myeloid DC alone, which is the main subject of our research, but applies also to other types of antigen-presenting cells (APCs). We use autoimmunity to the CNS-specific myelin–oligodendrocyte glycoprotein (MOG) as a representative example. The pathogenic role of MOG in EAE has been reviewed elsewhere [3].

A primary lesion hypothesis

Conforming to Wilkin's 'primary lesion hypothesis', we regard multiple sclerosis (MS) as being caused by a 'genetically predisposed high responsiveness to the excess of myelin released by an antecedent pathological event within the CNS' [4]. Because the brain is a hostile environment for T cells, promoting massive apoptosis, it is unlikely that autoreactive T and B cells are primed there [5,6]. However, we have recently shown in EAE-affected monkeys and MS patients that the T-cell areas in the cervical lymph nodes (CLNs), which drain the brain, are enriched with myelin-containing antigen-presenting cells (APCs), indicating that at this site, priming of new autoreactive T cells might take place [7].

Any damage to an organ, such as trauma, virus infection or stroke, can potentially break tolerance. However, this usually does not happen unless a (genetically determined) high responsiveness to the released self-antigens renders the affected individual prone to develop an autoimmune disease. For years, genetic predisposition has been interpreted as resulting from the interaction between MHC susceptibility elements with certain epitopes of self-antigens. Indeed, actively induced tolerance to the myelin basic protein can limit the CNS damage by cerebral ischemic injury [8]. However, the current insights into the function of innate receptors on APCs force us to include innate immunity in our concepts on the regulation of immune reactivity to self-antigens.

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Box 1. C-type lectins

The receptor repertoire of antigen-presenting cells (APCs) is extensive, recognizing a wide range of proteins, saccharides, lipids and nucleic acid ligands of endogenous, as well as exogenous, origin [56] (Table I). Most C-type lectin receptors (CLRs) function as antigen capture and clearance receptors that are involved in antigen capture and presentation [57]. CLRs mediate endocytosis, which is guided by their intracellular domains, which contain leucine-or tyrosine-based or a tri-acidic cluster, as internalization motifs. Some CLRs contain ITIM (immunoreceptor tyrosine-based inhibitory motif) or ITAM (immunoreceptor tyrosine-based activating motif) motifs in their cytoplasmic tail, illustrating potential immunosuppressive or immunoactivation functions of these receptors on APCs [12]. CLRs possess different numbers of carbohydrate recognition domains (CRDs), ranging from the expression of a single domain [e.g. DC-SIGN (DC-specific intercellular adhesion molecule 3-grabbing non-integrin), Dectin, DCIR (dendritic-cell immunoreceptor)] to a sequence of up to ten different CRDs (e.g. DEC205). The specificity of the CLRs for a specific glycan structure is currently being explored. Some CLRs recognize N-linked glycosylations, whereas others recognize O-linked glycosylations. Moreover, although some CLRs have specificity for single mannose, fucose or galactose structures, others recognize more complexed sugar moieties; their specificity is determined by multivalency, branching of the carbohydrates as well as the protein backbone of the glycoprotein that exposes the carbohydrate structure. O-linked structures are often exposed on collagens and mucins and on pathogens, whereas N-linked structures are present on the vast majority of the glycoproteins in the body, as well as on pathogens that use host-glycosylation for their survival and spread, such as retroviruses [20].

CLRs are considered to be antigen receptors that enhance antigen presentation by APCs. However, the specific antigens have not been well defined for all CLRs. Some CLRs have been well characterized as pathogen receptors [20] or adhesion and signaling receptors that bind self-glycoproteins on specific subsets of cells [12]. However, we think that the biological function of CLRs is the recognition of glycosylated self-antigens and that this function is misused by pathogens to escape a specific immune reaction [11]. For example, the mannose receptor recognizes lysosomal hydrolases, certain collagen-like peptides in serum and thyroglobulin (a well known self-antigen) and might also have a role in autoimmunity. The fact that all pathogens that target the CLR DC-SIGN inhibit a specific immune reaction [20], illustrates that CLR tolerance to self-glycoproteins might be induced in this way [11].

Table I. Human C-type lectins involved in self-glycoprotein recognition

CLR	Expression	Function	Self-glycoprotein	Pathogen binding
DEC205 (CD205)	DCs, macrophages, TECs	Inhibitory	Unknown	Unknown
MR (CD206)	DCs, macrophages, Ecs	Inhibitory	Lysosomal hydrolases, thyroglobulin, L-selectin	HIV-1, zymosan, bacteria, *M. tuberculosis*, yeast
DC-SIGN (CD209)	DCs, Hoffbauer cells, perivascular macrophages	Inhibitory	ICAM-2, ICAM-3, MAC-1 (PMN)	HIV-1, viruses, bacteria, *M. tuberculosis*, yeast, parasites
L-SIGN (CD209b)	LSECs, LNECs	ND	ICAM-2, ICAM-3	HIV-1, viruses, bacteria
MGL	DCs, macrophages	ND	Tumor MUC2	Unknown
ASGP-R	Hepatocytes	ND	Lutropin	Unknown
Endo180	Macrophages, ECs, fibroblasts	Inhibitory	Collagen, uPAR	Unknown
DCAL	DCs, macrophages	ND	Ligand on T cell	Unknown
Dectin	DCs, macrophages, granulocytes	Activating	Ligand on T cell	Microbes, zymosan

Abbreviations: ASGP-R, asialoglycoprotein receptor; CLR, C-type lectin receptors; DCAL, dendritic cell-associated lectin; DCs, dendritic cells; DC-SIGN, DC-specific intercellular adhesion molecule 3-grabbing non-integrin; ECs, endothelial cells; ICAM-2, intercellular adhesion molecule-2; LNECs, lymph node endothelial cells; LSECs, liver sinusoidal endothelial cells; L-SIGN, liver/lymph node-SIGN; MAC-1, macrophage-1 (CD11b); MGL, macrophage galactose/N-acetylgalactosamine-specific lectin; MR, mannose receptor; *M. tuberculosis*, *Mycobacterium tuberculosis*; MUC, mucin; ND, not done; PMN, polymorphonuclear cell; TECs, thymic endothelial cells; uPAR, urokinase-type plasminogen activator.

Homeostatic control of APC activity by CLR–TLR crosstalk

T cells with high affinity for self-antigens are deleted from the repertoire in the thymus early in life. Thymic selection is leaky, however, because T cells with a low affinity for self-antigen or those specific for self-antigens that do not access the thymus (such as several CNS antigens) can escape. To control autoreactivity, the immune system uses regulatory T (Tr) cells [9]. Two main types of Tr cells have been identified: (i) natural Tr or suppressor T (Ts) cells, which reside in the thymus and seem to exert their suppressive effect mainly by cell–cell contact and (ii) adaptive Tr or Tr1 cells, which seem to operate in peripheral lymphoid organs through suppressive cytokines, such as interleukin-10 (IL-10) and transforming growth factor-β (TGF-β) [9] There is increasing evidence that Tr1 cells can be induced by *in vitro*-generated immature DCs (iDCs), which lack co-stimulatory molecules (CD40, CD80/86) and fail to produce cytokines, such as IL-12 [10]. The *in vivo* counterparts of *in vitro*-generated iDCs have not been identified yet but might include blood monocytes or resident APCs in tissues (e.g. perivascular microglia cells in the brain). This implies that the control of DC and APC maturation is a crucial condition for the maintenance of tolerance.

The induction of DC maturation by pathogens involves a crosstalk between two antigen receptor families, CLRs and TLRs [11]. CLRs are transmembrane proteins, which specifically recognize carbohydrate structures on glycoproteins. The family of CLRs expressed on mammalian APCs (macrophages, DCs and endothelial cells) contains at least 15 known members [12] (Box 1). The *in vivo* localization of CLRs hints at an important function in self-antigen clearance and tolerance induction (Box 1). For example, the CLR DC-SIGN (DC-specific intercellular adhesion molecule 3-grabbing non-integrin) is highly expressed by DCs in placenta at the interface of mother–child antigen transmission, a site where immune tolerance has a central role. DC-SIGN is also highly expressed in the spleen on sinusoidal cells that form a direct contact between blood and tissue. Furthermore, this CLR is prominently expressed at various mucosal sites, in the brain and by lymph node DCs located in the T-cell area.

The TLR family is highly conserved in evolution and constitutes nine (rodents) or 10 (humans) known

members, which function as receptors for conserved molecular patterns on pathogens, pathogen-associated molecular patterns (PAMPs) [13]. TLRs not only recognize pathogen structures but might also interact with self- or conserved proteins, such as heat shock proteins (e.g. TLR4) [13]. TLRs are crucial receptors for the activation of innate immune mechanisms [14]. Several groups have recently reported the crosstalk of TLRs and CLRs as a regulatory mechanism of APC function [15–19].

The impact of the CLR–TLR crosstalk on the stimulatory capacity of DCs is illustrated by experiments with mycobacteria, which activate DCs through TLR2 and TLR4 and induce Th1 responses. However, some *Mycobacterium* strains can suppress DC activation by the secretion of CLR-binding factors [e.g. ManLAM (mannose-capped lipoarabinomannan)] [20].

Disturbed CLR–TLR crosstalk might cause autoimmunity in MS

In healthy individuals, tolerance to self-antigens is maintained by the balanced crosstalk between CLRs, which sample self-antigen and induce tolerance, and TLRs, which sense danger signals by PAMPs and induce immune activation. Box 2 (and Figure I therein) show that the normally balanced CLR–TLR crosstalk can be disturbed by overstimulation of TLRs and/or understimulation of CLRs, leading to DC maturation, and ultimately autoimmunity.

The infection paradigm implies strengthening of the TLR signal, although the strength of the CLR signal does not essentially change. In an experimental setting, tolerance to self can be disrupted by simultaneous administration of self-antigen and strong TLR stimuli. Although unaware of this principle, immunologists have used it for decades in the creation of autoimmune disease models in animals, by immunization with self-antigen and adjuvant. Recent experiments in outbred groups of non-human primates show that the more severe clinical and neuropathological presentation of EAE in rhesus monkeys compared to marmosets might be more dominated by the TLR response to bacterial antigens in the inoculum than to the MHC background [21]. Seminal experiments in mice have shown that antigens, such as ovalbumin (OVA) [22] or MOG peptide 35–55 [23], targeted to the CLR DEC-205 *in vivo* are efficiently presented to T cells and induce systemic tolerance in the absence of DC maturation, but induce systemic immunity in the presence of agonistic anti-CD40 antibody (a DC maturation signal) [24]. Similarly, DC maturation signals through CpG ligation to TLR9 can also break tolerance to self-antigen and induce autoimmune disease [25].

The alternative possibility, that autoimmunity is caused by understimulation of CLRs, has received little attention. One of the conditions in which inadequate stimulation of CLRs can occur is when the glycosylation of self-antigens is greatly disturbed. This altered glycosylation paradigm might explain why autoimmunity can become chronic.

Self-antigen glycosylation and autoimmunity

Proteins are glycosylated in the secretory pathway on their way to the cell surface, either to be expressed as a cell surface protein or to be released into the extracellular milieu [26]. Protein glycosylation is a highly complex process that involves >100 glycosyltransferases and glycosidases. The fact that ~1% of the mammalian genome encodes factors involved in glycan production and modification underscores the physiological importance of protein glycosylation [27]. Formation of most glycoproteins is initiated within the endoplasmatic reticulum, usually by the covalent linkage of the N-glycan N-acetylglucosamine to asparagin (N-glycosylation) or of the O-glycan N-acetylgalactosamine to serine or threonine (O-glycosylation). The shaping of the specific carbohydrate epitopes by glycosyltransferases, glucosidase- and mannosidase-dependent processing takes place in the endoplasmatic reticulum and in the Golgi system [27]. Glycosylation of self-proteins is also a dynamic process that varies in space and time. Some glycosyltransferase genes are ubiquitously expressed throughout the body, whereas others are differentially expressed among body tissues [28]. Moreover, glycan structures change in association with cellular metabolism and/or differentiation induced by cytokines, hormones and, importantly, in the context of autoimmune diseases, with pregnancy, stress and ageing [26].

The rheumatoid factors (RFs) in rheumatoid arthritis (RA) are an example of autoimmunity due to a change in the normal glycosylation of self-antigen. RFs are antibodies directed towards the Fc tail of serum IgG, which in RA often lacks one or both terminal galactose residues in their hinge region glycans due to defective β1–4 galactosyltransferase activity [29]. A similar deficiency expressed during active experimental arthritis in DBA/1 and MRL–*lpr*/*lpr* mice [30,31], renders anti-collagen autoantibody more pathogenic, possibly by the increased capacity of agalactosyl IgG antibodies to fix complement [32]. The spontaneous arthritis developing in ageing DBA/1 and MRL-*lpr*/*lpr* mice might possibly be caused by the defective glycosylation of self-glycoprotein [33,34].

Also in MS, aberrant glycosylation of CNS proteins has been reported. A comparative analysis in 42 MS patients and 20 patients with other neuropathies revealed reduced lectin-reactivity of cerebrospinal fluid glycoproteins as a probe for glycosylation [35]. Moreover, the fact that the activity of β–1,6-N-acetylglucosaminyltransferase V (Mgat5), a key enzyme in the N-glycosylation pathway, is decreased by 25–30% in MS patients when compared to healthy controls, is highly interesting [36]. Mice deficient for this enzyme are characterized by increased T-cell reactivity and increased susceptibility to EAE compared to wild-type mice [37]. The authors provide solid evidence for a profound effect of the enzyme deficiency on T-cell receptor (TCR) clustering, however, this does not explain the spontaneous glomerulonephritis observed in the knockout animals. This autoimmune phenomenon in Mgat5-deficient mice might be caused by disturbed glycosylation of an unknown self-antigen. Unfortunately, no information on spontaneous inflammation in other organs in which Mgat5 is expressed, such as the brain, was given [28].

Box 2. C-type lectin receptor (CLR)–Toll-like receptor (TLR) balance and autoimmunity

Yin and Yang are the Taoistic symbols of the dialectic elements in nature, such as light versus dark, warm versus cold and weak versus strong (http://www.chinesefortunecalendar.com/yinyang.htm). For a healthy life, Yin and Yang should be in balance. We have chosen the Yin–Yang principle as a metaphor of the regulation of tolerance and immunity to self-antigens by dendritic cells (DCs).

DCs express TLRs and CLRs. TLRs bind to pathogen-associated molecular patterns (PAMPs) expressed on viruses and bacteria. Self-antigens, such as heat shock proteins (HSPs), or cell debris can also cause TLR stimulation. CLRs recognize specific glycan structures present on pathogens and self-glycoproteins (Box 1). The crosstalk between CLRs (Yin) and TLRs (Yang) determines whether DCs remain immature and induce tolerance, or mature and induce autoimmunity [11].

Steady state paradigm

The steady state paradigm is shown in Figure I (Healthy model; CLR and TLR in balance). In a resting immune system, DC maturation is inhibited. Maturation signals via TLRs are balanced (buffered) by inhibitory signals via CLR recognition of self-glycoproteins. Tolerance is maintained by immature DCs, which process and present self-glycoproteins to induce regulatory T (Tr) cells. Tr cells keep autoreactive T cells (and possibly also the DC itself [58]) inactive.

Infection paradigm

The infection paradigm is shown in Figure I (Infection model; CLR and TLR temporarily out of balance). Disturbance of the normal CLR–TLR balance by infection or activation of a latent virus induces DC maturation **(a)** Mature DCs capture self-glycoproteins and present these in the context of co-stimulation molecules (CD80/86) and proinflammatory cytokines to autoreactive T and B cells. When the infection is cleared, the buffer capacity of the CLR ascertains that the CLR–TLR balance is restored **(b)** As a consequence, the steady-state control of immunity to self-glycoproteins is restored and disease remission is induced. Persistent viral infection of the central nervous system (CNS), however, causes chronic autoimmune encephalomyelitis, such as in mice infected with Theiler's murine encephalomyelitis virus [59].

According to the infection paradigm, the release of self-antigens from damaged myelin, due to stroke or infection, does not automatically lead to chronic autoimmune disease. Experimental autoimmune disease models induced by immunization, such as experimental autoimmune encephalomyelitis (EAE), are based on this infection model. Most EAE models are self-limiting because they are induced in normal laboratory animals. The disease goes into remission once the TLR-stimulating effect of the adjuvant has waned.

Post-translational alteration paradigm

The post-translational alteration paradigm is shown in Figure I (Altered glycosylation model; CLR and TLR permanently out of balance). This model describes how abnormal glycosylation of CLR ligands, for example, self-glycoproteins, can put individuals at risk of chronic autoimmune disease. DC maturation induced by infection or exacerbation of latent virus infection **(a)** causes autoimmunity, as described earlier in the infection model. However, after clearance of the infection **(b)**, the CLR–TLR balance will remain disturbed as a result of a deficient Yin input through CLRs. During this condition, tolerance to self is not restored. This model predicts that a relatively harmless TLR trigger in healthy individuals can become a risk signal in individuals with deficient buffering capacity by the CLR. The model also explains the spontaneous autoimmunity and enhanced susceptibility to specific autoimmune diseases of Mgat5-deficient mice [37].

Is MOG a protective antigen for the brain?

The CNS specificity of the autoimmune reactions in MS has focused the attention of many researchers on MOG. MOG is exclusively expressed in CNS myelin but its function is not known. Mice deficient for MOG exhibit no apparent structural abnormalities in their CNS myelin, suggesting that, in contrast to MOG or the two glycoproteins of peripheral nervous system myelin P0 and PMP-22, MOG has no direct function in the myelination of axons or structural stabilization of myelin [38,39]. By computer modeling, MOG has been depicted as a homodimer of two Ig-like molecules and the localization of the glycosylation, complement binding and antibody-binding regions in MOG were predicted [40]. MOG doublets are possibly complexed in the myelin membrane with galactocerebroside [41].

It is important for this discussion to distinguish between MOG in its natural conformation ('native MOG') and the *Escherichia coli* translated recombinant protein MOG1–125 (rhMOG) that is often used for experiments in animals (unglycosylated rhMOG). Native MOG has a single Ig-like extracellular domain, which contains the only N-linked glycosylation site at Asn31; O-linked carbohydrates have not been found. Native MOG is probably differentially glycosylated with N-linked oligosaccharides of the complex type, most of which express the L2/HNK-1 epitopes, which are present on several neural adhesion molecules, such as myelin-associated glycoprotein (MAG), L1 and NCAM (neural cell adhesion molecule) [42,43]. However, unlike these glycoproteins, MOG has no confirmed neural adhesion function.

Interestingly, T cells from native MOG-deficient mice exhibit a higher reactivity to rhMOG and a stronger capacity to transfer EAE than T cells from wild-type mice [44]. The most logical explanation is that thymus-derived Tr cells specific for native MOG are lacking in the MOG-deficient mice. However, it is unclear whether central tolerance to MOG exists because in wild-type mice MOG protein has not been found outside the CNS during thymic education. Moreover, MOG protein in the CNS is only expressed late in development, namely after postnatal day 10 in the mouse spinal cord and from day 17 in the brain [45]. There is some evidence that MOG transcripts are produced in the mouse thymus [46] or in rodent and primate Schwann cells [47] as consequence of promiscuous gene expression but expression of the protein has not been demonstrated. Notably, neuropathological changes in rhMOG-immunized marmosets remain remarkably confined to CNS myelin [48].

These data might imply that the control of MOG-reactive T cells in the naïve repertoire depends completely on peripheral tolerance mechanisms. Linares *et al.* [44] demonstrate that the presence of MOG in its natural conformation and embedded in myelin contributes to the control of T-cell autoreactivity. Indirect evidence for the presence of MOG-specific Tr cells in the peripheral compartment of marmosets has been reported [2].

Anti-MOG autoimmunity in non-human primate EAE models

The localization of MOG on the outer lamellae of the myelin sheaths and on the surface of oligodendrocytes

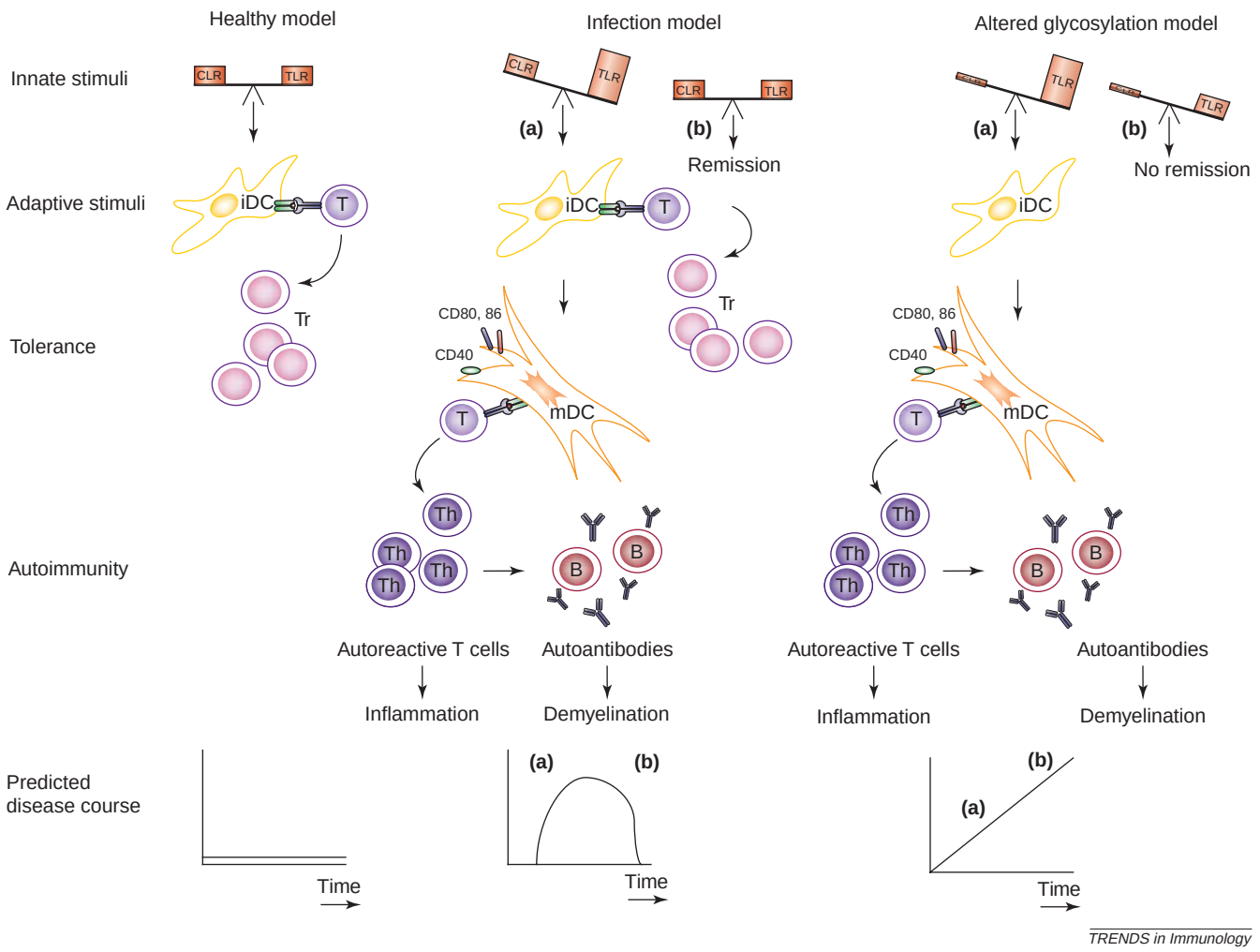

Figure I. Yin–Yang regulation of tolerance and autoimmunity by dendritic cells (DCs). Abbreviations: CLR, C-type lectin receptor; iDC, immature DC; mDC, mature DC; TLR, Toll-like receptor; Tr, regulatory T.

renders the antigen directly accessible to APCs, T cells and antibodies. MOG has therefore received much attention as a potential target of the autoimmune attack in MS.

Unglycosylated rhMOG is a remarkably strong autoantigen. With minute amounts of recombinant rhMOG, severe autoimmune encephalomyelitis can be induced in 100% of common marmosets (50–100 μg per monkey) and 100% of rhesus macaques (300–400 μg per monkey), despite the outbred character of both species. This might be due to the remarkably high frequency of rhMOG-reactive T cells in the naïve repertoire of marmosets, which has been estimated at 2.5 per 10^5 blood mononuclear cells [2]. The data suggest that this high frequency of autoreactive cells is kept under the strict control of Tr cells.

Marmosets immunized with human myelin, containing 'native' glycosylated MOG, or alternatively with unglycosylated rhMOG, exhibit remarkably different patterns of anti-MOG T- and B-cell autoimmunity. Monkeys immunized with the unglycosylated rhMOG protein display a variable diversification of the T-cell and antibody reactivity with a panel of 22mer peptides spanning the extracellular domain of human MOG [48,49]. T-cell lines against rhMOG and MOG peptides can easily be generated. IgG antibodies recognize conformational as well as linear MOG epitopes. By contrast, MOG-reactive T cells in myelin-immunized monkeys respond only to rhMOG and a narrow set of MOG peptides. Moreover, rhMOG-reactive T-cell lines seem to collapse after two or three rounds of stimulation with rhMOG, and immune sera contain IgG antibodies to conformational epitopes but lack detectable reactivity with linear epitopes (B.A. 't Hart, unpublished). These data support the concept that 'native' glycosylated MOG might be a protective factor in EAE and demonstrate that unglycosylated rhMOG is a much more potent trigger of a specific cellular and humoral autoimmunity than 'native' MOG.

To directly test whether 'native' MOG is protective we have immunized marmosets with myelin isolated from wild-type or MOG-deficient C57BL/6 mice [39]. Monkeys immunized with wild-type myelin containing 'native' MOG, remained free of clinical and neuropathological signs of EAE, whereas in monkeys immunized with MOG-deficient myelin, mild EAE developed (B.A. 't Hart, unpublished).

Box 3. Outstanding questions on altered glycosylation as a pathogenic factor in multiple sclerosis (MS) and experimental autoimmune encephalomyelitis (EAE)

Does the C-type lectin receptor (CLR)–Toll-like receptor (TLR) balance regulate autoimmune encephalomyelitis?
- The severity of EAE in non-human primates is associated with the TLR response to adjuvant.
- Targeting of xenoantigen [ovalbumin (OVA)] or self-antigen to the CLR DEC205 induces tolerance in mice. Tolerance is reversed by TLR9 occupancy with CpG and/or CD40 engagement.
- Mannosylated proteolipid protein (PLP)139–151 induces tolerance to EAE induced with this peptide in SJL mice.

Is self-glycosylation altered in MS?
- Cerebrospinal fluids of MS patients show a lower level of ConA-binding material than cerebrospinal fluids of other neuropathies.
- Glycosyltransferase (Mgat 5) activity in peripheral blood mononuclear cells is reduced 30% in MS patients.
- Glycosyltransferase (Mgat 5) knockout mice are more susceptible to EAE.

Is alteration of self-antigen glycosylation a pathogenic factor in EAE?
- Unglycosylated human myelin–oligodendrocyte glycoprotein (MOG)1–125 is a strong autoantigen that induces progressive broadening of the cellular and humoral immune responses in rodents and primates.
- Primates immunized with human myelin develop a narrower repertoire of immune response.
- Although myelin from wild-type mice is not encephalitogenic, myelin from MOG-deficient mice induces clinical and neuropathological signs of EAE in primates.

It is tempting to speculate on the basis of these data that tolerance to myelin antigens in healthy individuals is maintained by Tr cells, which are induced by iDCs, which are sampling glycosylated myelin antigens via their CLRs.

Pregnancy and tolerance

The ideal experiment to prove our concept in humans would be to reconstitute an autoimmune patient with immature 'tolerogenic' DCs to test whether reinforcement of tolerance and remission of disease symptoms takes place. This experiment has actually been performed by nature itself in pregnancy. It is well known that disease activity in MS is suppressed during pregnancy [50]. Pregnancy can be regarded as a temporary state of specific tolerance because the immune system of the mother is completely competent to combat pathogens but tolerates the antigens from the fetus [51]. The iDCs in the decidua, which have an important role in the maintenance of tolerance towards the fetus [51], clearly express CLRs, such as DC-SIGN [52,53] and L-SIGN (liver/lymph node-specific intercellular adhesion molecule-3-grabbing integrin) [54]. We hypothesize that the iDCs in the decidua maintain tolerance to the fetus by the continuous sampling of fetal glycoproteins leaking through the placenta by their CLR. It is tempting to speculate that at the same time they sample self-glycoproteins in the blood, reinforcing tolerance. Predictably, MS exacerbates after delivery of the child and the placenta, as a source of immature 'tolerogenic' APCs, is rejected.

Concluding remarks

Our concept further refines the danger hypothesis formulated by Polly Matzinger [55]. A healthy immune system will only be activated by pathogens that can tip the CLR–TLR balance by strong TLR stimulation. However, when the buffer capacity of CLRs is weakened, relatively harmless pathogens can cause immune activation (Box 3).

An important lesson from our concept is that the strengthening of the CLR signal-induced targeting with self-antigen, in combination with a therapy that prevents APC maturation, might be a feasible way to reinforce tolerance in autoimmune patients. We have given several examples showing that this principle works in rodent models. Future preclinical tests in non-human primates might predict the success of such a therapy in humans.

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