CMLS, Cell. Mol. Life Sci. 55 (1999) 1610–1625
1420-682X/99/121610-16 $ 1.50 + 0.20/0

CMLS Cellular and Molecular Life Sciences

Review

Interleukin-12, a key cytokine in Th1-mediated autoimmune diseases

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Received 15 January 1999; received after revision 11 March 1999; accepted 16 March 1999

Abstract. Interleukin 12 (IL-12) is a heterodimeric cytokine produced primarily by antigen-presenting cells (APCs) which plays a key role in promoting type 1 T helper cell (Th1) responses. The powerful activity of IL-12 requires tight control, which is exerted at various levels. Primary control is exerted on IL-12 production by APCs, a major factor driving the response towards the Th1 or Th2 phenotype. Another level of control regulates expression of the IL-12 receptor (IL-12R), which is composed of two subunits, $\beta 1$ and $\beta 2$. The IL-12R $\beta 2$ subunit has signal-transducing capacity and modulation of its expression is central to the regulation of IL-12 responsiveness. Endogenous IL-12 plays an important role in host defense against infection by a variety of intracellular pathogens. Its Th1-promoting activity, however, also favors Th1-mediated immunopathology and, in particular, the induction of Th1-mediated autoimmune diseases.

Key words. Interleukin-12; Th1; Th2; autoimmune diseases; insulin-dependent diabetes mellitus; NOD mice; IL-12-deficient mice.

The Th1/Th2 dichotomy

$CD4^+$ T cells can be distinguished, based on their pattern of cytokine production, into three major types: Th1, Th2 and Th0 [1, 2]. Th1 cells are characterized by secretion of interferon-γ (IFN-γ), and they mainly promote cell-mediated immunity able to eliminate intracellular pathogens, and the synthesis of complement-fixing antibody isotypes. Conversely, Th2 cells selectively produce interleukin (IL)-4 and are involved in the development of humoral immunity protecting against extracellular pathogens. Type 1 cytokines, associated primarily with Th1 responses, include in addition to IFN-γ, IL-2, IL-15 and tumor necrosis factor (TNF)-β. Type 2 cytokines, associated mainly with Th2 responses, include IL-5, IL-6, IL-10, and IL-13, in addition to IL-4. Th0 cells, which could either represent precursors of Th1/Th2 cells or a terminally differentiated subset, are not restricted in their lymphokine production.

The development of Th1 and Th2 cells is influenced by several factors, but three are most important: ligand-T cell receptor (TCR) interactions, genetic polymorphism, and cytokines (table 1). Decisive roles in the polarization of T cells are played by IL-12 and IL-4, guiding T cell responses towards the Th1 or Th2 phenotype, respectively [3, 4]. These, however, are not the only cytokines involved in the polarization of T cell responses; IL-18 and IL-13, for example, also play a role [5]. The avidity of interaction between peptide-class II ligands and the TCR is an important factor controlling the cytokine secretion profile. As demonstrated using different antigen doses in vitro [6, 7] and in vivo [8, 9] and by altered peptide ligands [10], lower-avidity interactions appear to favor Th2 cell development. The decision of naive $CD4^+$ T cells to develop into Th1 or Th2 effector cells is not simply related to whether the priming conditions include high levels of inflammatory cytokines such as IL-12. In the *Leishmania major* model, it

has been suggested that the T cell response which occurs in the absence of strong pathogen-driven signals may reflect a strain-specific intrinsic propensity to develop $CD4^+$ cells along the Th1 or Th2 pathway [11]. Genetic differences have been demonstrated between T cells of BALB/c versus B10.D2 mice in their Th phenotype acquisition under neutral condition in vitro, suggesting that the susceptibility of BALB/c mice to *L. major* infection may reside, at least in part, in their inability to sustain IL-12-dependent Th1 development rather than in the intrinsic capacity of BALB/c T cells to differentiate towards the Th2 phenotype [12]. The difference in maintenance of IL-12 responsiveness between BALB/c and B10.D2 T cells in vitro and the subsequent Th1/Th2 development is controlled by a single dominant genetic locus named T cell phenotype modulator 1 (*Tmp-1*) Although the precise gene(s) involved has yet to be determined, this locus has been mapped to a region of chromosome 11 containing a cluster of genes important for T cell differentiation, including IL-4, IL-5, IL-3, and other genes, such as interferon regulatory factor-1, that may influence Th1/Th2 development [13]. This region is syntenic with the homologous gene cluster in human chromosome 5 previously linked to several phenotypic markers of atopy.

Polarized Th1 and Th2 subsets can be generated from $CD4^+$ populations in vitro [14], can be recovered from primed animals [15] and are found in patients suffering from autoimmune or allergic diseases [16]. However, polarized Th1 and Th2 cells represent extremes in a spectrum. Detection of intracytoplasmic cytokine production by polarized Th1 and Th2 cell populations analyzed at the single-cell level has confirmed the existence of defined Th1 and Th2 cells, selectively producing IFN-γ or IL-4, respectively, but has also revealed intermediate patterns [17]. Within this spectrum, discrete subsets of differentiated T cells secreting a mixture of Th1 and Th2 cytokines, for example IFN-γ and IL-10, have been identified [18].

Molecular mechanisms to explain the polarization of Th1 and Th2 subsets, based on the differential expression of the receptors for IFN-γ and IL-12, do exist. The ability of IFN-γ to inhibit the proliferation of Th2 but not Th1 cells may be related to the lack of IFN-γ receptor (IFN-γR) β chain expression in Th1 cells [19]. However, IFN-γR β chain loss also occurs in IFN-γ-treated Th2 cells, and therefore does not appear to represent a Th1-cell-specific differentiation event [20]. Conversely, developmental commitment to the Th2 lineage results from rapid loss of IL-12 signalling in Th2 cells [12]. The inability of Th2 cells to respond to IL-12 appears to be due to selective down-regulation of the IL-12R β2 subunit [21–23]. Inhibition of Th1 and induction of Th2 in vivo is also related to down-regulation of IL-12R β2 subunit expression [24]. These findings are therefore consistent with a general model in which selective modulation of IL-12 signalling plays an important role in the acquisition of polarized Th cell phenotypes (fig. 1). Modulation of chromatin structure also regulates cytokine gene expression during T cell differentiation [25]. Differentiation of naive T helper cells into mature Th2 cells is associated with chromatin remodelling of the IL-4 and IL-13 genes, whereas differentiation into Th1 cells involves selective remodelling of the IFN-γ gene. IL-4 locus remodelling is accompanied by demethylation and requires both antigen stimulation and Stat6 activation.

The reciprocal regulation between Th cell subsets is another driving force polarizing $CD4^+$ T cells into differentiated Th1 or Th2 cells. IL-12 promotes the development of Th1 cells [14, 26, 27] and inhibits IL-4-induced IgE synthesis [28]. IFN-γ amplifies the IL-12-dependent development of Th1 cells [29] and inhibits Th2 cell proliferation [30]. Conversely, IL-4 and IL-10 inhibit lymphokine production by Th1 clones [31]. In addition, IL-10 [32], IL-4, and IL-13 [33] suppress the development of Th1 cells through down-regulation of IL-12 production by monocytes. However, the reciprocal regulation of IL-12 and IL-4 is not only negative. For example, IL-12 administered to mice after the establishment of a *L. major*-specific Th2 response actually enhances rather than suppresses IL-4 production [34, 35].

Table 1. Factors affecting Th1/Th2 development (MHC, major histocompatibility complex; APC, antigen-presenting cell).

- Cytokines (IL-12, IL-4, IFN-γ, IL-18, IL-10, IL-13)
- Avidity of MHC-peptide/TCR interaction
  - APC type
  - mode of antigen administration
  - dose of antigen
  - affinity of peptide-MHC binding
  - costimulation
- Genetic background

IL-12 production by antigen-presenting cells

IL-12 is a 75-kDa heterodimer composed of two covalently linked glycosylated chains, p35 and p40, encoded by distinct genes [36, 37]. This cytokine, produced predominantly by activated monocytes and dendritic cells but also by other cell types such as neutrophiles [38], enhances proliferation and cytolytic activity of natural killer (NK) and T cells, and stimulates their IFN-γ production [39]. Most importantly, IL-12 induces the development of Th1 cells in vitro [14, 26] and in vivo [27]. In addition, IL-12 is a potent cofactor stimulating growth, IFN-γ synthesis, and cell adhesion of already

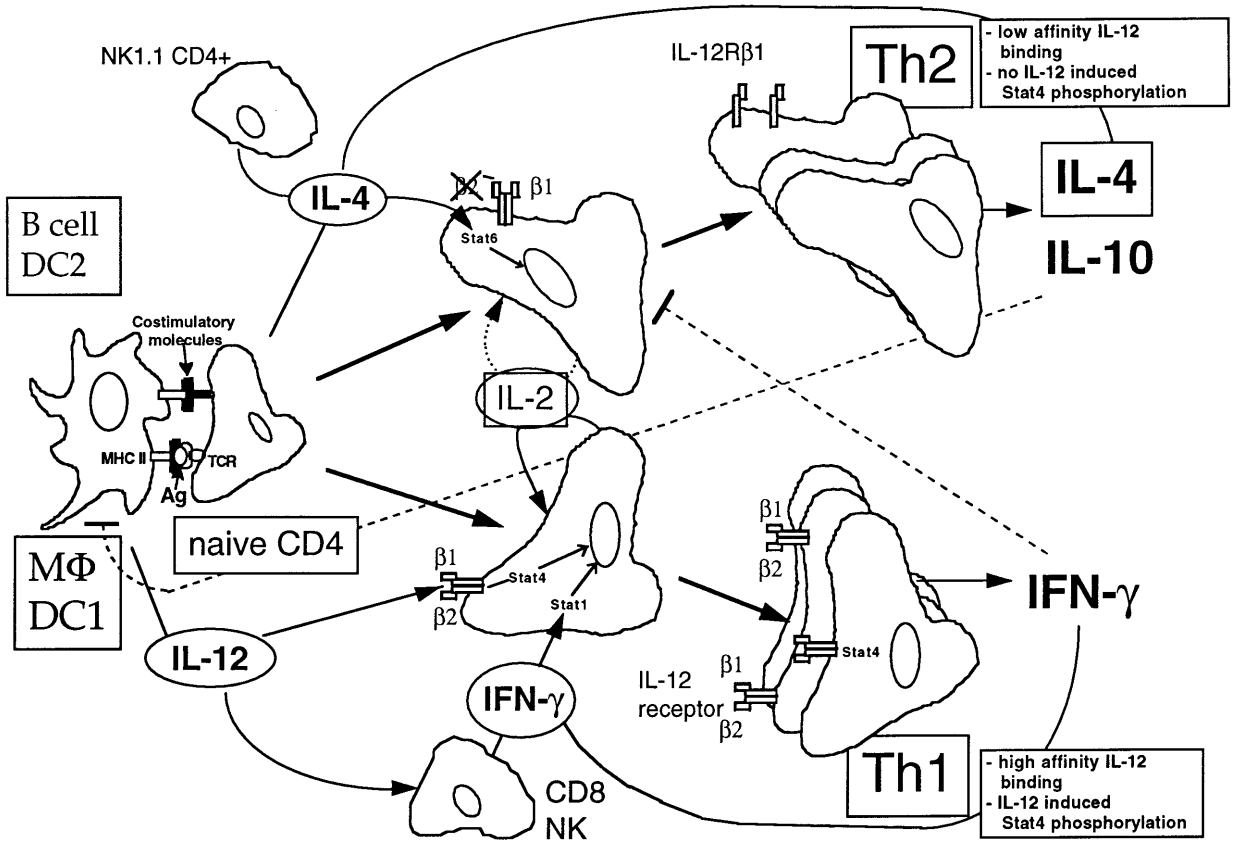

Figure 1. Role of IL-12 in the regulation of Th1/Th2 cell development. Activation of naive T cells through triggering of the antigen receptor is sufficient for the initial expression of functional IL-12 receptors. Depending on the cytokines present in the microenvironment, T cells will progressively develop into IL-12-responsive Th1 or IL-12-non-responsive Th2 cells. IL-12 and IL-4 act through Stat4 and Stat6, respectively, to deliver specific differentiation signals. Due to loss of IL-12R $\beta 2$ chain expression, Th2 cells extinguish IL-12 responsiveness. The principal sources of IL-12 are activated macrophages (MΦ) and DC1 cells, whereas IL-4 is initially produced by antigen-stimulated $CD4^+$ T cells and $CD4^+NK1,1^+$-type T cells. IL-4 produced by mast cells and basophils may also contribute to Th2 cell development [compiled from refs 3, 21, 22, 24].

differentiated Th1 cells [40]. Thus, IL-12 is a key cytokine in immunoregulation [3]. The powerful activity of IL-12 requires tight control, which is exerted at various levels. The primary control is exerted on IL-12 production by antigen-presenting cells (APCs), a major factor driving the response towards the Th1 or Th2 phenotype.

Normal mature mouse B cells present antigen to $CD4^+$ T cells but fail to produce IL-12: selective APCs for Th2 cell development?. Among the different APC populations capable of presenting peptide-class II MHC complexes, dendritic cells (DCs) and B cells have been studied extensively, but their relative role in the presentation of protein antigen and $CD4^+$ T cell priming in vivo is still controversial. Using mice lacking B cells, presentation to $CD4^+$ T cells of antigenic complexes derived from processing of protein antigen administered in adjuvant was found in some studies to require B cells [41], whereas in others, B cells were found to be not critical [42]. Similarly, administration of soluble protein to normal mice led to selective expression of antigenic complexes either by antigen-specific B cells [43] or by DCs [44].

To address this point, we have compared the relative capacity of DCs and B cells, recruited in lymph nodes during the inflammatory response induced by adjuvant administration, to present protein antigen administered in different forms. Immune lymph node cells (LNCs) from mice immunized with hen egg-white lysozyme (HEL) in adjuvant display HEL peptide-MHC class II complexes able to stimulate, in the absence of any further antigen addition, specific T hybridoma cells [45, 46]. Using this read-out system, we have compared expression by DCs and B cells of antigenic complexes derived from processing of native HEL, either given subcutaneously in adjuvant or in soluble form intra-

venously (i.v.). Following subcutaneous administration of HEL in adjuvant, DCs are the only APC expressing detectable HEL peptide-class II complexes [47]. Conversely, when HEL is administered in soluble form i.v. to mice previously injected with adjuvant only, lymph node B cells are much more efficient than DCs in the presentation of HEL peptides [48]. These results demonstrate that protein antigen injected in soluble form is presented best by B cells, whereas the same protein is presented only by lymph node DCs when administered in adjuvant. Therefore, different protocols of protein antigen administration lead to expression of peptide-class II complexes by different APCs.

Although IL-12 was discovered as a product of Epstein-Barr virus (EBV)-transformed B cells [49, 50], secretion of IL-12 by normal B cells is still unclear. B cells, even when activated to induce similar levels of T cell proliferation as DCs, still drive a Th0-type response, with relatively low levels of IFN-γ being produced even upon neutralization of IL-4 [7]. The low level of IFN-γ produced by Th cells does not appear to be IL-12 dependent, suggesting that normal B cells do not produce this cytokine [51]. In fact, B cells have been shown to inhibit IL-12 production [52]. Conversely, other MHC class II+ APCs have been shown to be major producers of IL-12. Among them, macrophages secrete high levels of p75 when stimulated by bacteria such as *Staphylococcus aureus* or by bacterial products such as lipopolysaccharide (LPS) plus IFN-γ [53]. In addition, macrophages have been shown to direct, via secretion of IL-12, the development of antigen-specific T cells towards the Th1 phenotype [14]. Macatonia et al. [54] have shown IL-12 production by DCs, and IL-12 p75 is secreted by DCs upon contact with T cells [55–57].

We have studied antigen presentation and IL-12 production ex vivo by APCs obtained from an inflammatory site, since IL-12 is likely to be produced in higher amounts at the site of inflammation by professional APCs. DCs were found to be unique among APCs in the capacity to present efficiently peptide-class II complexes formed in vivo and to simultaneously secrete substantial amounts of IL-12 p75 [58]. IL-12 secretion by DCs is strongly up-regulated by ligand-TCR and CD40-CD40L interactions with $CD4^+$ T cells, which are established through cognate interaction during antigen presentation. The feedback from antigen-specific T cells leading to increased IL-12 secretion is much more efficient in DCs than in macrophages, in contrast to bacterial stimuli which induce similar levels of IL-12 secretion in both cell types. Interestingly, at least two DC subsets exist: myeloid-like cells (DC1) produce abundant IL-12 and induce a Th1 response, whereas lymphoid-like cells (DC2) induce a Th2 response without secreting IL-4, implicating a unique signal provided by DC2 cells in the differentiation towards the Th2 phenotype [59]. The efficient presentation of antigenic complexes derived from proteins present in inflammatory sites and the production of IL-12 may account for the capacity of DCs recruited in immune lymph nodes to prime naive $CD4^+$ T cells in vivo. We have shown that IL-12 administration to non-obese diabetic (NOD) mice accelerates autoimmune diabetes, associated with massive infiltration of lymphoid cells, including DCs, into the pancreas [60]. Considering the efficiency of DC1 in antigen presentation and their capacity to produce IL-12, it is likely that pancreatic DC1-type cells play a role in the induction of diabetogenic Th1 cells [61] and therefore may represent an interesting target to treat organ-specific autoimmune diseases.

Conversely, mouse spleen and lymph node B cells fail to secrete IL-12 following either antigen-specific interaction with T cells or non-specific stimulation [58]. The capacity of B cells to present antigen in vivo, at least under some conditions [48, 62], but not to secrete IL-12, may play a role in selectively priming Th2 cells. Several lines of evidence suggest that antigen presentation by B cells may skew the development of $CD4^+$ cells towards the Th2 pathway [63–65]. Induction of experimental allergic encephalomyelitis (EAE), a Th1-mediated autoimmune disease, has been prevented by targeting the autoantigen to B cells [66], and this prevention is associated with the priming of antigen-specific Th2 cells [67]. IgD targeting on the B cell surface by bivalent antibody fragments results in B cell activation [68], which might be a prerequisite for priming of Th2 cells [67]. In addition, lack of spontaneous recovery from EAE by B-cell-deficient mice has been attributed to the absence of B cells capable of driving Th2 responses [69]. Therefore, antigen presentation by APCs lacking the capacity to secrete IL-12, such as B cells, could favor Th2 development. This hypothesis is in agreement with the observation that antigen presentation by B-cell-enriched populations, instead of monocytes, induced much greater IL-4 synthesis in $CD4^+$ cells from allergic individuals [70]. Since B cells, in vivo, can present protein more effectively than peptide antigen to $CD4^+$ T cells [43], this may also explain why soluble protein but not peptide administration diverts the immune response of TCR-transgenic $CD4^+$ cells to the Th2 pathway [71]. However, a subset of human tonsillar B cells has been recently shown to secrete low levels of bioactive IL-12 [72]. It is still unclear whether this reflects a general difference between human and mouse B cells or whether lack of IL-12 production by B cells is specific to BALB/c mice, in which Th2 development is favored.

Selective induction of Th2 cell responses by APCs unable to secrete IL-12 is not restricted to B cells. Keratinocytes, the most abundant cell type in the skin, when activated express class II molecules and can function as APCs. Activated T cells stimulated by keratinocytes, in

contrast to professional APCs, produce almost exclusively Th2-type cytokines and very little IFN-γ. This immune deviation appears to result from the inability of keratinocytes to secrete IL-12, because IFN-γ production is restored by exogenous IL-12 [73].

Thus, autoantigen presentation by IL-12-deficient APCs could effectively induce immune deviation to the Th2 phenotype. It would be interesting to evaluate antigen targeting to non-IL-12-producing APCs in the treatment of established Th1-mediated autoimmune diseases.

Th1 cells induce and Th2 cells inhibit antigen-dependent IL-12 secretion by DCs. DCs have been demonstrated to be the most relevant source of antigenic peptide-MHC class II complexes in lymph nodes draining inflammatory sites [47]. Their high presenting activity, together with the display of costimulatory molecules have been suggested as the molecular basis for their ability to prime naive T cells [42, 74, 75]. DCs are unique among lymph-node-derived APCs because they present antigen efficiently [76] and secrete IL-12 [54–58, 77, 78], a potent Th1-driving cytokine.

We have studied Th1- and Th2-mediated regulation of IL-12 secretion by lymph nodes DCs [79]. Upon interaction with antigen-specific T cells, DCs expressing appropriate peptide-class II complexes secrete IL-12, a cytokine driving Th1 cell development. To analyze the T-cell-mediated regulation of IL-12 secretion by DCs, we have examined their capacity to secrete IL-12 in response to stimulation by antigen-specific Th1 and Th2 DO11.10 TCR-transgenic cells. Th1 cells induce while Th2 cells inhibit IL-12 secretion by DCs, in both cases via antigen-specific interactions. Induction of IL-12 secretion by Th1 cells is not mediated by Th1-derived IFN-γ but requires cell surface contact between Th1 cells and DCs via class II/peptide-TCR and CD40-CD40L interactions. Triggering of CD40 by CD40L-expressing T cells had been previously found to regulate IL-12 production by monocytes in response to antigen but not to LPS [80]. The inhibition of IL-12 secretion by Th2 cells is mediated by antigen-induced soluble factor(s) including IL-10. These data imply that an ongoing Th1 response will generate a positive feedback loop by inducing DCs expressing specific peptide-MHC complexes to secrete IL-12. Conversely, a negative loop for IL-12 secretion is generated by Th2 cells. This loop is dominant over the positive one.

These observations suggest that antigen-specific polarized Th1 and Th2 cells can influence immune responses by regulating IL-12 secretion. The regulation of IL-12 production by differentiated Th1 and Th2 cells could also play an important role in established immune responses. IL-12 is not only a differentiation factor essential for the development of Th1 cells, but is also a costimulus for activation and growth of effector Th1 cells [40, 81]. Evidence for this is also provided by the observation that anti-IL-12 antibodies can inhibit established Th1-mediated autoimmune colitis [82] and the pathogenic effect of proteolipid protein (PLP)-specific Th1 cells in an adoptive transfer model of EAE, when administered after cell transfer [83]. Thus, the capacity of Th2 cells to inhibit, mainly via IL-10, Th1-induced IL-12 production could contribute significantly to the inhibition of ongoing IL-12-dependent autoimmune diseases. This may represent an additional mechanism explaining the inhibitory effect of Th2 cells in Th1-mediated autoimmune diseases.

Astrocytes inhibit IL-12 secretion by central nervous system microglia: regulation of IL-12 secretion by APC-APC interaction. To elucidate mechanisms regulating IL-12 production in an immune-privileged site such as the central nervous system (CNS), recent studies have investigated IL-12 production by two CNS glial cell populations, microglia and astrocytes. Microglia, CNS APCs morphologically and functionally related to the monocyte/macrophage lineage, synthesize and secrete heterodimeric IL-12 [84, 85]. Conversely, astrocytes, CNS APCs of neuroectodermal origin, fail to produce IL-12 p75 and secrete only minimal amounts of p40 molecules [85]. Interestingly, astrocytes actively suppress IL-12 secretion by microglia [85]. These findings demonstrate a novel mode of IL-12 regulation at the APC level in the absence of T cells.

Since IL-12 promotes IFN-γ secretion by T cells [86] and IFN-γ in turn stimulates antigen presentation by microglia [87], it is likely that this cytokine network provides a positive feedback loop for induction of Th1 responses and microglia activation during infection or inflammation. The primary reason for heterodimeric IL-12 secretion by microglia is probably to promote immune responses against neurotropic infectious agents. IL-12 can exert its protective activity by stimulating NK and T cells, and inducing IFN-γ secretion by these cell types [88]. As a consequence of intracerebral IL-12 production, an immunostimulatory loop that perpetuates chronic inflammation within the brain can be established via induction of Th1 cells. Microglia, which restimulate efficiently both Th1 and Th2 cells, secrete IL-12 upon antigen-dependent interaction with Th1, but not with Th2 cells [89]. Th1-driven IL-12 production depends on TCR ligation by antigen, CD40 engagement on microglia, and IFN-γ secretion by activated Th1 cells. Th1 and, to a lesser extent, Th2 cells also stimulate the production of prostaglandin E_2 (PGE_2) by microglia [90]. T-cell-induced PGE2 secretion requires MHC class II-peptide/TCR interactions but does not depend on CD40 engagement or on the presence of IFN-γ. As PGE_2 can inhibit Th1 responses via down-regulation of IL-12 production by APCs [91], including microglia [85], and IL-12R expression [92], it

may represent a negative feedback mechanism able to limit the propagation of Th1 responses.

The anti-inflammatory cytokine IL-10 is also a powerful inhibitor of microglial IL-12 production, both at the mRNA and protein level [85]. This parallels the capacity of IL-10 to inhibit IL-12 production as well as other costimulatory surface molecules (e.g., CD80/CD86) or soluble cytokines (e.g., TNF-α, IL-1β) by professional APCs such as macrophages and DCs [32, 81, 93]. Microglia are the major intracerebral source of IL-10, suggesting an additional autocrine negative feedback mechanism able to interrupt an immunostimulatory circuit. Prevention of EAE by IL-10 administration [94], increase of IL-10 mRNA and IL-10-expressing cells in the brain during the recovery phase of active EAE [95], and the presence of an IL-10/IL-12 regulatory circuit controlling susceptibility to EAE [96] are consistent with a role for IL-10 in down-regulating CNS inflammation.

Astrocytes and microglia differ markedly in their ability to provide costimulatory signals for T cell activation [97]. In addition, unlike microglia, astrocytes fail to secrete IL-12 [85]. In this respect, astrocytes are similar to non-professional APCs such as keratinocytes, which fail to secrete IL-12 and promote Th2 rather than Th1 responses [73]. IFN-γ-activated mouse astrocytes can process and present antigen and, quite interestingly, are very efficient in stimulating Th2-type cytokine secretion [89]. The finding that astrocytes inhibit IL-12 p75 secretion by IFN-γ/LPS-stimulated microglia suggests a possible mechanism for inhibition of Th1 responses by astrocytes [85]. Astrocytes also down-regulate secretion of TNF-α by LPS-stimulated microglia, but do not reduce or even increase IL-10 secretion by microglia. These data raise the possibility that astrocytes inhibit synthesis of proinflammatory cytokines while enhancing deactivating mechanisms in microglia. This, in conjunc-

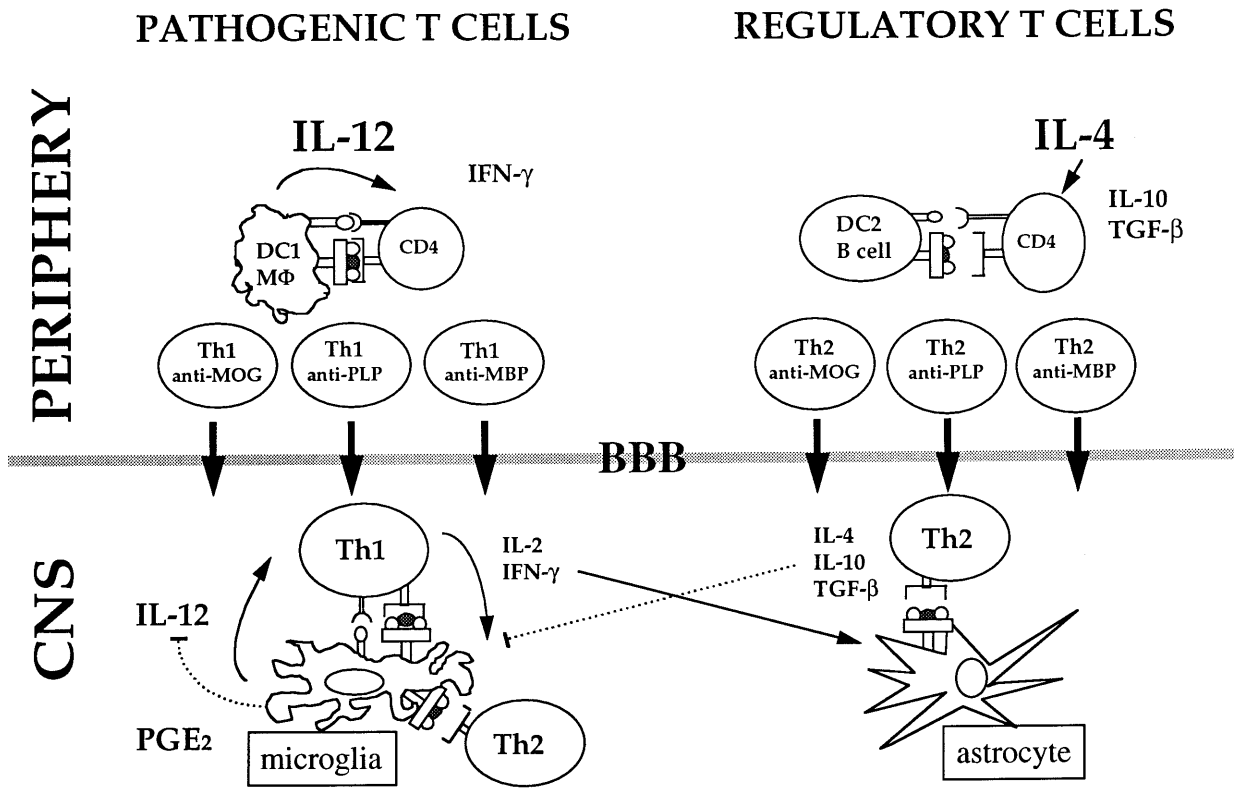

Figure 2. Pathogenic and regulatory T cell subsets in EAE/MS. $CD4^+$ T cells primed in peripheral lymphoid organs cross the blood-brain barrier (BBB) and secrete Th1- and Th2-type cytokines upon recognition of the target antigen, e.g. myelin basic protein (MBP), proteolipid protein (PLP) and myelin oligodendrocyte glycoprotein (MOG), on perivascular and resident CNS APCs. Within the CNS parenchyma, microglia are the main cell type expressing MHC class II and adhesion/costimulatory molecules and they can release several mediators (IL-12, IL-10, TGF-β, PGE_2, chemokines, among others) regulating T helper phenotype, recruitment, and activation. Astrocytes may also be recruited into the inflammatory network to secrete immunoregulatory mediators (e.g. colony-stimulating factors, PGE_2, chemokines) in response to T-cell-derived (IFN-γ) and microglia-derived (IL-1, TNF-α) cytokines, but their APC capacity is limited to Th2 restimulation in the presence of antigenic peptides.

Table 2. Autoimmune diseases mediated by Th1 cells.

- Rheumatoid arthritis
- Insulin-dependent diabetes mellitus
- Experimental allergic encephalomyelitis/multiple sclerosis
- Autoimmune thyroiditis
- Inflammatory bowel disease
- Uveoretinitis
- Myasthenia gravis

tion with inhibition of IL-12 secretion, may limit spreading of inflammation in the brain parenchyma (see Fig. 2).

Th1 and Th2 cells in autoimmune diseases

The relative role of Th1 and Th2 cells in autoimmune diseases is currently a major topic of research. At present, the results indicate a critical role for Th1 cells in the pathogenesis of many organ-specific autoimmune diseases. Conversely, the role of Th2 cells is still unclear, although indirect evidence for their protective capacity has been provided.

Experimental models. Th1 cells are considered to be involved in the induction of several experimental autoimmune diseases [61, 98–100] (table 2). Evidence for this is based on adoptive transfer experiments demonstrating that $CD4^+$ cells producing Th1-type lymphokines can transfer disease, both in EAE [101] and in insulin-dependent diabetes mellitus (IDDM) [102–104] models. However, cytokine regulation is complex; for example, TNF-α and IL-10 have opposite effects on IDDM depending on the developmental stage of the immune system [105, 106]. This could also explain why, in some cases, β cell destruction in IDDM has been associated with Th2 rather than Th1 cells [107, 108].

The reciprocal regulation between T cell subsets predicts a role for Th2 cells in inhibition of autoimmune diseases. Regulatory T cells that suppress the development of EAE produce Th2-type cytokines [109] and recovery from EAE is associated with increased Th2 cytokines in the CNS [110]. In addition, administration of IL-4 to mice with EAE ameliorates the disease [63]. These results clearly suggest that activation of Th2 cells may prevent EAE. A role for Th2 cells has also been proposed for inhibition of IDDM development. Evidence for a protective role of Th2 cells is provided by the reduced IDDM incidence following IL-4 [111] or IL-10 [112] administration to NOD mice. A role for Th2 cells regulating the onset of IDDM is also suggested by their capacity to inhibit the spontaneous onset of diabetes in rats [113, 114] and by the correlation between protection from IDDM and IL-4 production in double-transgenic mice on a BALB/c background [115]. Transgenic NOD mice that express IL-4 in their pancreatic β cells are protected from insulitis and IDDM, a direct indication that Th2 cytokines can prevent destructive autoimmunity [116].

However, Th2 cells transgenic for a TCR derived from a clone able to transfer IDDM, when injected into neonatal NOD mice, invaded the islets but neither provoked disease nor provided substantial protection [104]. Similar results were also obtained by adoptive transfer of non-transgenic Th1 and Th2 cell lines into neonatal mice [117]. Therefore, these data do not support the concept that Th2 cells afford protection from IDDM, at least in the effector phase of the disease. Rather, they are in accord with the observation that transgenic expression by islet cells of IL-10 [106, 118], an inhibitory lymphokine of Th1 cells, actually promotes insulitis and IDDM.

Collectively, these results point to a critical role of Th1 cells in the induction of autoimmune diseases, whereas the influence of Th2 cells is still controversial. In any case, whether or not Th2 cells exert a direct protective role, diversion away from proinflammatory Th1 cells should effectively reduce the chronic inflammatory response which is typical of organ-specific autoimmune diseases.

Human diseases. Th1 cells appear to be involved in human organ-specific autoimmune diseases also (table 2). $CD4^+$ T cell clones isolated from lymphocytic infiltrates of Hashimoto's thyroiditis or Graves' disease exhibit a clear-cut type 1 phenotype [119]. In addition, most T cell clones derived from peripheral blood or cerebrospinal fluid of multiple sclerosis (MS) patients show a Th1 lymphokine profile [120]. Expression of IL-12 p40 mRNA has been detected in acute MS lesions, particularly from early disease cases [121], suggesting that IL-12 up-regulation may be an important event in disease initiation. T cells from MS patients induce CD40L-dependent IL-12 secretion in the progressive but not in the relapsing-remitting form of the disease, suggesting a link to disease pathogenesis [122, 123].

Involvement of Th1 cells has also been suggested in other human autoimmune diseases. Insulitis in IDDM patients has been shown to comprise a large number of IFN-γ-producing lymphocytes [124]. T cell clones derived from the synovial membrane of rheumatoid arthritis (RA) patients also display a Th1 phenotype as they produce, upon activation, large amounts of IFN-γ and no or very little IL-4 [125]. Another study has shown that most $CD4^+$ and $CD8^+$ clones recovered from synovial fluid of RA patients display a Th1 phenotype [126]. Interestingly, in situ hybridization for T cell cytokine expression demonstrates a Th1-like pattern in most synovial samples from RA patients, whereas samples from patients with reactive arthritis, a disorder with

similar synovial pathology but driven by persisting exogenous antigen, express a Th0 phenotype [127].

The situation is less clear in most systemic autoimmune disorders. In general, heterogeneous cytokine profiles are found in the serum or target organs of patients with systemic autoimmunity, such as systemic lupus, Sjögren's syndrome, and primary vasculitis [16].

The role of IL-12 in Th1-mediated autoimmune diseases

The important and non-redundant role of IL-12 in the induction of Th1 responses has been demonstrated in mice deficient for IL-12 [128], IL-12Rβ1 [129], or Stat4 [130]. Mice that develop a Th1-type response to *L. major* are resistant to infection but in the absence of IL-12 mount a polarized Th2 cell response and succumb to infection [131]. IL-12-deficient mice also fail to control mycobacterial infections due to a decreased ability to develop Th1-mediated protective immunity [132]. Similarly, humans with genetic deficiency for IL-12 or IL-12R demonstrate systemic dissemination of otherwise poorly pathogenic bacteria [133–135]. In contrast, IL-12 deficiency does not alter the control of viral infections, indicating that alternative pathways for the generation of type 1 responses may be induced.

It has been hypothesized that IL-12 differentially regulates the effector response in infectious and autoimmune diseases and that in the latter case it may function independently of IFN-γ [136]. At any rate, a pathogenic role of Th1 cells is clearly documented in several autoimmune disease models such as EAE, collagen-induced arthritis (CIA), experimental autoimmune uveitis (EAU), and experimental autoimmune myasthenia gravis (EAMG) [reviewed in refs 100, 137].

Insulin-dependent diabetes mellitus. Administration of IL-12 induces rapid onset of IDDM in 100% of NOD female mice, whereas only about 60–70% of control littermates eventually develop IDDM [60]. This effect is not due to toxicity of IL-12 for pancreatic β cells, as shown by the normal appearance of islet cells and by the absence of IDDM in BALB/c mice treated with IL-12. Acceleration of IDDM in genetically susceptible NOD mice is accompanied by increased Th1 cytokine production by islet-infiltrating $CD4^+$ and $CD8^+$ T cells, and by selective destruction of islet β cells, suggesting a causal link between IL-12, Th1 cell induction, and development of IDDM.

To study the role of Th1 and Th2 cells in IDDM, we targeted endogenous IL-12 in NOD mice by administration of the IL-12 antagonist $(p40)_2$ [138]. Administration of $(p40)_2$ from 3 weeks of age, before the onset of insulitis, results in the deviation of pancreas-infiltrating $CD4^+$ but not CD8+ cells to the type 2 phenotype and in the reduction of spontaneous and cyclophosphamide-accelerated IDDM. After treating NOD mice with $(p40)_2$ from 9 weeks of age, when insulitis is well established, few Th2 and a reduced percentage of Th1 cells are found in the pancreas. This is associated with a slightly decreased incidence of spontaneous IDDM but, at variance with a recent report [139], no protection from cyclophosphamide-accelerated IDDM. $(p40)_2$ can inhibit in vitro the default Th1 development of naive TCR-transgenic $CD4^+$ cells to the Th2 pathway but does not modify the cytokine profile of polarized Th1 cells, although it prevents further recruitment of $CD4^+$ cells into the Th1 subset. When polarized Th1 cells infiltrate the pancreas, targeting endogenous IL-12 has a marginal effect on IDDM incidence. This implies that inhibition of IL-12 may not inhibit pathogenic differentiated Th1 cells in chronic progressive diseases such as IDDM, whereas it could be beneficial in remitting/relapsing diseases such as EAE or some forms of MS. In conclusion, the immune deviation to Th2 is maximal when IL-12 is targeted before the onset of insulitis, and is associated with protection from IDDM.

Experimental allergic encephalomyelitis. IL-12 administration significantly increases the severity of EAE [140]. Similarly, mice treated with IL-12 in vivo following the transfer of PLP-stimulated LNCs develop a more severe and prolonged form of EAE compared to vehicle-treated controls. Most importantly, administration of anti-IL-12 antibodies substantially reduces the incidence and severity of adoptively transferred EAE, suggesting that endogenous IL-12 plays a key role in its pathogenesis [83]. In addition, IL-12-deficient mice are completely protected from EAE [96]. Consistent with its role in promoting the activation and differentiation of pathogenic Th1 cells, IL-12 was detected in the brain of rats with EAE just before the development of clinical signs [95]. IL-12 can promote EAE via IFN-γ-dependent as well as -independent pathways, consistent with the separate but complementary roles of IL-12 and IFN-γ in the regulation of IL-12Rβ2 expression on antigen-specific $CD4^+$ cells [96]. B10.S mice fail to up-regulate the IL-12Rβ2 subunit and are resistant to EAE. The defective expression of IL-12Rβ2 transcripts was not secondary to the production of suppressive cytokines, but to a failure of B10.S myelin basic protein (MBP)-specific T cells to up-regulate CD40L expression and to induce the production of IL-12 [141].

Collagen-induced arthritis. Treatment of DBA/1 mice with IL-12 enhances the autoimmune response to type II collagen, resulting in severe, destructive CIA. IFN-γ production by collagen-specific $CD4^+$ T cells as well as synthesis of complement-fixing antibodies of IgG2a and IgG2b isotypes is strongly up-regulated, suggesting that IL-12-induced Th1 cells may play a crucial role in the pathogenesis of this form of arthritis [142]. This conversion by IL-12 of a weak autoimmunogenic stimulus to a

strong one inducing severe arthritis is associated with a pronounced anti-type II collagen humoral immune response as well as dramatically enhanced type-II-collagen-specific IFN-γ production by $CD4^+$ T cells. This suggests that IL-12 unmasks latent autoimmunity by inducing Th1 cells which most likely have a crucial role in the pathogenesis of this particular form of arthritis. This view is confirmed by the reduced incidence of CIA in IL-12-deficient DBA/1 mice, although a few mice developed severe disease despite a highly reduced Th1 response [143]. Surprisingly, injection of high doses of IL-12 into DBA/1 mice with established CIA profoundly ameliorates disease [144]. It is possible that due to supraoptimal induction of IFN-γ by high endogenous levels of IL-12, a gene activation program involving regulation of apoptosis could be initiated, leading to apoptotic deletion of autoaggressive T cells. Suppressive effects of IL-12 treatment may also be mediated by a counter-regulatory circuit driven by IL-10.

Experimental colitis. Th1-mediated experimental colitis can be induced by rectal administration of the haptenizing reagent 2,4,6-trinitrobenzene sulfonic acid (TNBS) [82]. This disease can be treated by administration of anti-IL-12 antibodies even late after onset, suggesting that endogenous IL-12 may be required not only for induction but also for progression of experimental colitis [82]. Administration of anti-CD40L antibodies during the induction phase of the Th1 response prevents IFN-γ production by lamina propria $CD4^+$ T cells, and also clinical and histological evidence of disease. Disease prevention is caused by inhibition of IL-12 secretion, as demonstrated by immunohistochemistry [145]. Experimental colitis can also be inhibited by oral administration of haptenized colonic proteins (HCPs) before rectal administration of TNBS. This form of oral tolerance appears to be due to the generation of mucosal T cells producing transforming growth factor (TGF)-β and Th2-type cytokines. The suppressive effect of orally administered HCP is abrogated by the concomitant administration of anti-TGF-β or IL-12, suggesting a reciprocal relationship between IL-12 and TGF-β on tolerance induction in TNBS-induced colitis [146].

Autoimmune diseases in IL-12-deficient mice

Several studies have addressed the role of IL-12 in autoimmune diseases by using IL-12-deficient mice. In general, induced autoimmune diseases are reduced or absent in IL-12-deficient mice, whereas IDDM, a spontaneous Th1-mediated autoimmune disease, appears to develop in IL-12-deficient mice (table 3).

To evaluate the role of endogenous IL-12 in IDDM development, mice deficient in IL-12 were generated by targeted disruption of the gene encoding the p40 subunit [128] and backcrossed to the NOD background. IL-12-deficient NOD mice show profoundly reduced antigen-specific Th1 responses in draining lymph nodes and addition of IL-12 but not IL-18 restores Th1 development in vitro, demonstrating the non-redundant role of IL-12. Unexpectedly, IL-12-deficient NOD mice develop pancreas-infiltrating Th1 cells and autoimmune diabetes similar to controls. T cell recruitment in the pancreas seems favored in IL-12-deficient NOD mice as revealed by increased P-selectin ligand expression on pancreas-infiltrating T cells, and this can compensate for the defective Th1 cell pool recruitable from peripheral lymphoid organs. In addition, residual Th1 cells could accumulate in the pancreas of IL-12-deficient NOD mice because Th2 cells are not induced, in contrast to wild-type NOD mice treated with an IL-12 antagonist [S. Trembleau et al., unpublished data]. Therefore, targeting endogenous IL-12 does prevent IDDM [138], but its genetic absence does not. It is possible that in IL-12-deficient NOD mice, other cytokines may compensate for the lack of IL-12. A candidate potentially able to replace IL-12 could be the IFN-γ-inducing factor IL-18. A rise in both IL-18 and IL-12 p40 mRNA levels has been detected in the adherent cell population of cyclophosphamide-treated NOD mice [147]. IL-18 synergizes with IL-12 but is not able to restore the production of IFN-γ by HEL- or protein purified derivative (PPD)-specific T cells from IL-12-deficient NOD mice [S. Trembleau et al., unpublished data]. Thus, IL-18 only acts on IL-12-primed Th1-developing cells stimulating them to produce more IFN-γ, but in the absence of IL-12 is inefficient in inducing the differentiation of Th1 cells. These data are consistent with results indicating that IL-12 is sufficient for normal Th1 development in the absence of IL-18 [148], and that IL-18 by itself does not induce Th1 cell development [149, 150]. However, mice deficient in both IL-12 and IL-18 display a more profound impairment in the BCG-induced Th1 response compared with IL-12-deficient

Table 3. Th1-mediated autoimmune diseases in IL-12-deficient mice.

Absent
• Experimental allergic encephalomyelitis [96]
• Experimental autoimmune uveoretinitis [158]
Reduced
• Collagen-induced arthritis [143]
• Experimental autoimmune myasthenia gravis [157]
• Autoimmune thyroiditis [P. Zaccone et al., in press]
Unmodified
• Insulin-dependent diabetes mellitus [S. Trembleau et al., in press]

mice, suggesting that IL-12-independent Th1 development could be induced by the cooperative action of IL-18 and other factor(s), as yet unidentified [148]. This pathway could account for the residual Th1 development in IL-12-deficient NOD mice but accumulation of diabetogenic Th1 cells in their pancreata is likely to depend on alternative mechanisms.

A similar situation has been described for IFN-γ itself, a cytokine produced by Th1 cells which has been implicated in the effector mechanisms leading to β cell destruction. Inhibition of endogenous IFN-γ protects from disease [151, 152], but IDDM develops in IFN-γ-deficient NOD mice [153]. However, insulitis does not develop in IFN-γ receptor (IFN-γR)-deficient NOD mice [154]. In addition, development of insulitis and IDDM does not occur in IFN-γ-deficient RIP-LCMV-transgenic mice [155], admittedly a more artificial system in which to study autoimmune diabetes.

While there is no clear explanation at present for the discrepancy between IFN-γ- and IFN-γRα-deficient mice, it should be noted that it has also been observed in other models [15]. Recently, IFN-γRβ-deficient mice were found to differ from IFN-γRα-deficient mice in their ability to develop Th1 responses [156]. More detailed understanding of the IFN-γ signalling pathway may explain these seemingly conflicting results. In any case, the genetic absence of IL-12 or IFN-γ allows the development of compensatory mechanisms not available in unmanipulated NOD mice, in which IDDM can be prevented by treatment with cytokine antagonists.

The nature of the autoantigen(s) and the chronicity of IDDM combined with a genetic deficiency in immunoregulation could lead, even in the absence of IL-12, to a diabetogenic Th1 development in the NOD mouse. IL-12 deficiency consistently leads to decreased autoantigen-specific Th1 responses in induced autoimmune diseases such as CIA, EAMG, EAU, and EAE [96, 143, 157, 158] (table 3). However, the concomitant induction of Th2-type responses or other immunoregulatory pathways is variable. In CIA, IL-12 deficiency is not associated with a significant modification of IL-5 levels and IL-4 is still undetectable [143]. In EAMG, autoantigen-specific cells from IL-12-deficient mice produce mainly IL-4 [157]. In EAU, antigen-specific T cells from IL-12-deficient mice show increased production of IL-5 and IL-10 and no change in IL-4 levels [158], whereas they fail to secrete IL-10 or IL-4 in the EAE model [96]. In the latter case, an immunoregulatory circuit involving IL-10 produced by antigen non-specific $CD4^+$ cells has been described. Interestingly, IL-12-deficient mice are only partially protected from CIA and EAMG, whereas they appear to be completely protected from EAE and EAU. Altogether, these results suggest that impaired development of autoimmune Th1 cells may not be sufficient and the induction of an immunoregulatory pathway could be necessary for complete inhibition of an autoimmune disease. This regulation could depend more on IL-10 than IL-4, as indicated by the observation that IL-4-transgenic mice do develop EAE but IL-10-transgenic mice are completely protected [159], and by the capacity of IL-10-producing Tr1 cells to inhibit autoimmune colitis [160]. IL-12-deficient NOD mice show a major reduction of PPD- or HEL-specific IFN-γ production but little enhancement of IL-4 and IL-10 secretion. Likewise, very few pancreas-infiltrating T cells produce IL-4 or IL-10. A defective IL-4 production by NOD $CD4^+$ cells has been implicated in IDDM development [111] and it has been associated with an impairment in NK1.1 $CD4^+$ cells, which could be involved in early IL-4 production [161]. It is possible that immunoregulatory pathways involving IL-10 are impaired in NOD mice as well. Consistent with this assumption, administration of a non-cytolitic IL-10-fusion protein to NOD mice completely protects from IDDM [162]. The absence of IL-12 combined with a lack of immunoregulatory circuits, could still allow the development of autoimmune Th1 cells in a chronic progressive autoimmune disease under polygenic control, such as IDDM. Although IL-10 is clearly an immunosuppressive factor in the late phase of IDDM in NOD mice [112, 162], its presence before 2 weeks of age in NOD mice favors the generation of effector $CD8+$ T cells leading to pancreatitis and accelerated IDDM [163].

Overall, these results point to the fact that multiple mediators and effector mechanisms contribute to IDDM, and that disruption of genes encoding a single mediator may not necessarily affect the natural course of disease, as already noted for EAE [164]. The observation that IL-12 and IFN-γ are dispensable for IDDM development is consistent with the notion that $CD8+$ T cells, which are required for IDDM development in NOD mice, are unaffected in mice genetically deficient in IL-12 [128] or IFN-γ. In addition, it is likely that the genetic absence of IL-12 or IFN-γ allows the development of compensatory mechanisms not available in unmanipulated NOD mice, which do respond to treatment with cytokine antagonists. Conditional gene targeting, which offers the possibility to inactivate a gene at the desired time, should be able to clarify these issues.

The role of IL-12 in EAMG, an antibody-mediated disease

IL-12 has been clearly shown to be involved in the pathogenesis of Th1-mediated autoimmune diseases, but its role in antibody-mediated autoimmune pathologies has not been extensively addressed. We investigated

the effects of exogenous and endogenous IL-12 in EAMG [157]. EAMG is an animal model for myasthenia gravis (MG), a T-cell-dependent, autoantibody-mediated disorder of neuromuscular transmission caused by antibodies to the muscle nicotinic acetylcholine receptor (AChR). Administration of IL-12 with Torpedo AChR (ToAChR) to C57BL/6 (B6) mice resulted in increased ToAChR-specific IFN-γ production and increased anti-ToAChR IgG2a serum antibodies compared with B6 mice primed with ToAChR alone. These changes were associated with earlier and greater neurophysiological evidence of EAMG in the IL-12-treated mice, and reduced numbers of AChRs. In contrast, when IL-12-deficient mice were immunized with ToAChR, ToAChR-specific Th1 cells and anti-ToAChR IgG2a serum antibodies were reduced compared to ToAChR-primed normal B6 mice, and the IL-12-deficient mice showed almost no neurophysiological evidence of EAMG and less reduction in AChRs. These results indicate an important role of IL-12 in the induction of an antibody-mediated autoimmune disease, suggest that Th1-dependent complement-fixing IgG2a anti-AChR antibodies are involved in the pathogenesis of EAMG, and help to account for the lack of correlation between anti-AChR levels and clinical disease seen in many earlier studies.

It is possible that Th1 cells can directly attack the neuromuscular junction in IL-12-treated mice. IFN-γ-deficient mice were resistant to EAMG and this was associated with greatly reduced levels of both IgG1 and IgG2a antibodies specific for mouse AChR [165]. Conversely, transgenic expression of IFN-γ at the neuromuscular junction provoked an autoimmune humoral response resembling MG, characterized by infiltration of mononuclear cells and by autoantibody deposition at motor end plates, but these mice did not have detectable antibodies to mouse AChR [166]. In sera from our B6 mice injected with ToAChR and IL-12, however, levels of antibodies to ToAChR and to mouse AChR were raised, and IgG was bound to muscle AChRs consistent with a pathogenic role for antibodies. A possible explanation for the pathogenicity of IL-12-induced Th1 cells in EAMG, therefore, is that they induce increased production of complement-fixing anti-ToAChR IgG2a antibodies. IL-12 has been shown to up-regulate by two to three orders of magnitude the in vivo synthesis of complement-fixing IgG subclasses [167]. Complement-fixing anti-ToAChR antibodies could thus play an essential role in reducing the efficiency of neuromuscular transmission to an extent that results in d-tubocurarine-induced decrement. In mice, in contrast to humans, the safety factor for neuromuscular transmission is high and obvious weakness is only seen when the efficacy of transmission is severely impaired [168]. Therefore, in mice given IL-12, the extra deficit induced by complement-mediated damage to the neuromuscular junction must have been sufficient to uncover the defect in transmission. By contrast, in IL-12-deficient mice, the serum anti-ToAChR antibodies were predominantly of IgG1 isotype, only IgG1 was found at the neuromuscular junctions, and AChRs were not so reduced. Thus, the deficit in neuromuscular transmission correlates with the Th1-dependent IgG2a anti-ToAChR antibodies rather than with the Th2-dependent IgG1 antibodies. These results are important because they may explain the lack of correlation between antibody levels and clinical severity that has been reported in many previous studies of EAMG [168] which have not usually investigated IgG subclasses. EAMG can also be induced in mice by injection of purified mouse AChR without adjuvant [169]. It will be interesting to see whether this form of the disease is also associated with Th1-dependent IgG2a antibodies. In conclusion, EAMG involves an IL-12-dependent autoimmune response mediated by AChR-specific Th1 cells which promote the synthesis of pathogenic, complement-fixing, Th1-driven anti-AChR antibody isotypes. Targeting IL-12 may therefore be beneficial not only in T-cell-mediated [137] but also in antibody-mediated autoimmune diseases.

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