Introduction
CD4+ [1,2] and CD8+ [3] T-cells can be classified, on the basis of their pattern of cytokine production, into three major types: Th1, Th2 and Th0. The distinction between type 1 and 2 may indeed represent a fundamental dichotomy for all T-cell subsets [4]. Th1 cells are characterized by secretion of interferon γ (IFN-γ), interleukin (IL)-2 and tumour necrosis factor β (TNF-β); they promote cell-mediated immunity and are able to eliminate intracellular pathogens. Conversely, Th2 cells selectively produce IL-4 and IL-5, and are involved in the development of humoral immunity protecting against extracellular pathogens. 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 two are the most important: cytokines and ligand–T-cell receptor (TCR) interaction. Of the cytokines, IL-12 and IL-4 play decisive roles, guiding T-cell responses towards the Th1 or Th2 phenotype respectively [5,6].

Polarized Th1 and Th2 subsets can be generated from CD4+ populations in vitro [7], they can be recovered from primed animals [8] and they are found in patients suffering from autoimmune or allergic diseases [9]. However, polarized Th1 and Th2 cells represent the extremes of a spectrum. Detection of intracytoplasmic cytokine production by polarized Th1 and Th2 cell populations analysed 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 [10]. Within this spectrum, discrete subsets of differentiated T-cells secreting a mixture of Th1 and Th2 cytokines may exist, for example mouse T-cells secreting IFN-γ and IL-10 [11]. 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 lack of IFN-γ receptor β-chain expression in the latter [12]. However, IFN-γ receptor β-chain loss also occurs in IFN-γ-treated Th2 cells, and therefore does not appear to represent a Th1-cell-specific differentiation event [13]. Conversely, developmental commitment to the Th2 lineage results from rapid loss of IL-12 signalling in Th2 cells [14]. The inability of Th2 cells to respond to IL-12 may be due to down-regulation of the IL-12 receptor β2 subunit [15]. 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 distinct Th-cell phenotypes.

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 [7,16,17] and inhibits IL-4-induced IgE synthesis [18]. IFN-γ amplifies the IL-12-dependent development of Th1 cells [19] and inhibits Th2-cell proliferation [20]. Conversely, IL-4 and IL-10 inhibit lymphokine production by Th1 clones [21]. In addition, IL-10 [22], IL-4 and IL-13 [23] suppress the development of Th1 cells through down-regulation of IL-12 production by monocytes.

Th1 and Th2 cells in insulin-dependent diabetes mellitus (IDDM)
Th1 cells are thought to be involved in the induction of IDDM [24,25]. Evidence for this is based on adoptive transfer experiments demonstrating that CD4+ cells producing Th1-type lymphokines can transfer disease [26,27]. 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 [28,29]. This could also explain why, in some cases, β-cell destruction in IDDM has been associated with Th2 rather than Th1 cells [30,31].

The reciprocal regulation between T-cell subsets predicts a role for Th2 cells in inhibition of IDDM. Evidence for a protective role for Th2...
cells is provided by the reduced IDDM incidence after IL-4 [32] or IL-10 [33] administration to non-obese diabetic (NOD) mice. A role for Th2 cells in the regulation of the onset of IDDM is also suggested by their ability to inhibit the spontaneous onset of diabetes in rats [34] and by the correlation between protection from IDDM and IL-4 production in double-transgenic mice on Balb/c background [35]. 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 [36].

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 did not provoke disease and neither did they provide substantial protection [27]. Similar results were also obtained by adoptive transfer of non-transgenic Th1 and Th2 cell lines into neonatal mice [37]. 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 [29,38], an inhibitory lymphokine of Th1 cells, actually promotes insulitis and IDDM instead of inhibiting them.

Collectively, these results point to a crucial role for Th1 cells in the induction of IDDM, whereas the effect of Th2 cells is still unclear. In any case, whether or not Th2 cells exert a direct protective effect, diversion away from proinflammatory Th1 cells should be effective in reducing the chronic inflammatory response that is typical of organ-specific autoimmune diseases such as IDDM.

**Effect of exogenous IL-12 on IDDM development**

IL-12 is a heterodimer composed of two covalently linked glycosylated chains, p35 and p40, encoded by distinct genes [39,40]. This cytokine, produced predominantly by activated monocytes and dendritic cells but also by other cell types such as neutrophils, enhances proliferation and cytolytic activity of natural killer and T-cells, and stimulates their IFN-γ production [41]. Most importantly, IL-12 induces the development of Th1 cells in vitro [7,16] and in vivo [17]. In addition, it is a potent cofactor in the stimulation of growth, IFN-γ synthesis and cell adhesion of already differentiated Th1 cells [42].

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 the disease [43]. This effect is not due to the toxicity of IL-12 to the pancreatic β-cell, as shown by the normal appearance of the 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.

Conversely, by following a protocol developed by O'Hara and Henderson [44], we were able to confirm that intermittent administration of IL-12 (once weekly for 12 weeks) to NOD mice delays and reduces IDDM development (S. Trembleau, G. Penna, S. Gregori and L. Adorini, unpublished work). Data explaining these opposing effects of IL-12 are not yet available. However, it is conceivable that intermittent administration of IL-12, although still favouring Th1 induction, may be unable to sustain Th1-cell development. Thus the aborted induction of a Th1 response, possibly coupled to the emergence of regulatory Th2 cells, may result in delayed Th1-mediated disease progression. This result should not be considered a surprising paradox, but rather an expected property of Th1/Th2 cell regulation.

IL-12 may have a primary role in the induction of not only IDDM but also other organ-specific autoimmune diseases [25]. Given its crucial role in Th1-mediated autoimmune disease, IL-12 becomes an attractive target for immunointervention. As it is not only a differentiation factor essential for the development of Th1 cells, but also a co-stimulus for activation of effector Th1 cells [42,45], targeting IL-12 may represent a strategy not only to prevent but possibly also to treat Th1-mediated autoimmune diseases.

**Favouring the development of Th2 cells by IL-12 antagonist administration inhibits IDDM development**

Considering the powerful effects of IL-12 in vitro, it is likely that natural IL-12 antagonists may exist. One such antagonist could be the p40
molecule itself. The mouse p40 chain specifically antagonizes mouse IL-12 [46], and the primary inhibitory species is a disulphide-linked homodimeric form of IL-12 p40, termed (p40)2 [47]. On the basis of competitive binding assays performed under high-affinity-binding conditions, mouse (p40)2 appears to bind to the mouse IL-12 receptor with an affinity similar to that of IL-12, but it does not trigger biological activity and it specifically inhibits IL-12-mediated responses. The p40 homodimer is 25–50-fold more potent than the p40 monomer as an IL-12 receptor antagonist. This is paralleled by a similar capacity to inhibit IL-12-dependent activities, such as induction of IFN-γ synthesis by spleen cells. Human p40 also exists in dimeric and monomeric forms [48], and (p40)2 binds to a human T-cell line expressing high-affinity IL-12 receptor at least 20-fold more efficiently than the monomer. However, unlike mouse (p40)2, which binds to the mouse IL-12 receptor with an affinity comparable with IL-12 itself, human (p40)2 binds to the human IL-12 receptor with an affinity 5–10-fold lower than human IL-12, and human (p40)2 is correspondingly less potent than mouse (p40)2 in inhibiting IL-12 bioactivity. Collectively, the data available clearly demonstrate that both mouse and human (p40)2 bind to the IL-12 receptor and act as competitive antagonists of IL-12 function.

Evidence for p40 molecules being natural antagonists of IL-12 comes from the observation that p40 is produced in large excess over IL-12 both in vitro [49,50] and in vivo [51,52], and that p40 levels remain high for a long time after stimulation, whereas IL-12 production rapidly decreases [51,52]. After lipopolysaccharide (LPS) stimulation in vitro, mouse peritoneal exudate cells produce IL-12, p40 and (p40)2 molecules [25]. The naturally occurring (p40)2 can be visualized by Western-blot-analysis, purified and shown to inhibit IL-12-induced T-cell proliferation. Therefore it is conceivable that (p40)2 acts as an endogenous regulator of IL-12 activity. In addition to its ability to act as a competitive antagonist of IL-12 in vitro, mouse (p40)2 can also inhibit IL-12 functions in vivo, as demonstrated by decreased IFN-γ production after LPS administration to different mouse strains, including NOD, injected with (p40)2 (S. Trembleau, unpublished work).

To determine its capacity to affect Th1 and Th2 cell development, (p40)2 was injected daily into female NOD mice from 21 days of age, when no insulitis was yet present, until day 83. By 30 weeks of age, 90% of control mice but only 30% of mice treated with (p40)2 had developed IDDM. Intracytoplasmic staining of pancreas-infiltrating CD4+ cells from control mice revealed only IFN-γ-producing cells, up to 25%. Conversely, pancreas-infiltrating cells from (p40)2-treated mice contained up to 20% IL-4-producing cells in normoglycaemic mice but only 4% in diabetic mice. IFN-γ-producing cells were more abundant in the latter than in the former case. Thus protection from IDDM is associated with a clear-cut deviation to the Th2 phenotype.

The effect of (p40)2 was also studied in adult NOD mice, which display florid insulitis characterized by a high percentage of IFN-γ- and no IL-4-producing cells. NOD mice were treated continuously with (p40)2 from 9 weeks of age onwards. By 30 weeks of age, 79% of control mice and 54% of mice treated with (p40)2 had developed IDDM. Intracytoplasmic staining of pancreas-infiltrating CD4+ cells from (p40)2-treated mice never showed more than 4% of IL-4-producing cells. This would suggest that partial protection from IDDM is associated with partial deviation to the Th2 phenotype.

In conclusion, (p40)2 administration favours Th2 development, which is associated with protection from disease. This IL-12 antagonist, administered before the onset of insulitis, almost completely abrogates IDDM. However, treatment initiated after the onset of insulitis, when Th1 cells have already infiltrated the pancreatic islets, is less effective. Primed Th1 cells do not reverse their phenotype when restimulated by antigen in the presence of (p40)2, but their further recruitment is prevented, as demonstrated in a TCR transgenic system (S. Trembleau, G. Penna, S. Gregori, M.K. Gately and L. Adorini, unpublished work). We are currently trying to analyse whether Th2 cells are directly responsible for protection from IDDM or whether the immune deviation away from Th1 itself accounts for the decreased incidence of the disease.

Prospects

The results reviewed highlight the crucial role of Th1 cells in the induction of IDDM and suggest a possible effect of Th2 cells in controlling, directly or indirectly, disease induction and/or progression.

Regulation of the Th1/Th2 balance can be
effectively induced not only by cytokine targeting, but also by antigen administration. The Th1/Th2 paradigm has indeed emphasized immune deviation as a mechanism for cytokine- or antigen-based immunointervention in autoimmune diseases [53]. An example of immune deviation is provided by autoantigen-specific therapy of IDDM in NOD mice. Autoantigens in IDDM are fairly well known [54]. Glutamic acid decarboxylase (GAD) appears to be the most important, because responses to it are detected before responses to other autoantigens, including insulin, heat shock protein, peripherin and carboxypeptidase H [55,56]. Intravenous or intrathymic administration of GAD to 3-week-old NOD mice, which do not yet display islet infiltration, prevents T-cell proliferation to GAD in 12-week-old mice, and also prevents the development of intra-islet infiltration and IDDM in adult NOD mice. The reduction in the number of IFN-γ-secreting GAD-specific T-cells [55] associated with the continuing production of autoantibodies to GAD [56] suggests that parental GAD administration has induced a shift towards a Th2 response. Evidence for induction of a protective Th2 response has recently been obtained by nasal administration of GAD peptides to NOD mice [57]. These data indicate that nasal administration of GAD65 peptides induces a Th2 cell response that inhibits the spontaneous development of autoreactive Th1 responses and the progression of β-cell autoimmunity in NOD mice.

Collectively, these results suggest that immune deviation towards the Th2 phenotype may be effective in treating Th1-mediated autoimmune diseases, but the most effective manipulation of Th1 and Th2 cells in autoimmunity may eventually rely on a combination of antigen- and cytokine-based approaches. Ideally, they could be used to target specifically autoreactive T-cells, diverting them from autoaggression by changing their lymphokine-production profile. This strategy, which has been successfully applied to immunotherapy of parasitic diseases [17,58], may complement attempts at treating human autoimmune diseases based on self-antigen administration.

Basic and Clinical Aspects of Autoimmunity


Received 23 December 1996