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The Role of IL-12 in Autoimmune Diabetes

Introduction

The Th1/Th2 dichotomy has become a central paradigm in immunoregulation. The basic tenets of the Th1/Th2 paradigm are simple: $CD4^+$ T cells can be distinguished, based on their pattern of cytokine production, into three major types (Mosmann et al., 1986; Del Prete et al., 1991). Th1 cells are characterized by secretion of interferon-γ (IFN-γ), IL-2, and TNF-β, and they promote cell-mediated immunity able to eliminate intracellular pathogens. 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 or a terminally differentiated subset, are not restricted in their lymphokine production. A similar distinction applies to $CD8^+$ cells (Mosmann and Sad, 1996). The development of Th1 and Th2 cells is influenced by several factors, but three are most important: local cytokines, the avidity of ligand-TCR interaction and the non-MHC genetic polymorphism. 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 (Paul and Seder, 1994; Trinchieri, 1995). The strength of peptide/class II- TCR interaction, which depends on the overall avidity of APC-T cell interactions, also controls the profile of cytokine secretion by T cells. As demonstrated by using different antigen doses in vitro and in vivo as well as by altered peptide ligands, lower avidity interactions appear to favor Th2 cell development (Abbas et al., 1997).

Another driving force polarizing $CD4^+$ T cells into differentiated Th1 or Th2 is the reciprocal regulation between Th cell subsets. IL-12 promotes the development of Th1 cells and inhibits IL-4-induced IgE synthesis. IFN-γ amplifies the IL-12-dependent development of Th1 cells and inhibits Th2 cell proliferation. Conversely, IL-4 and IL-10 inhibit lymphokine production by Th1 clones. In addition, IL-10, IL-4 and IL-13 suppress the development of Th1 cells through downregulation of IL-12 production.

However, in reality, the situation is not so simple. Although polarized Th1 and Th2 subsets can be generated from $CD4^+$ populations in vitro (Hsieh et al., 1993), can be recovered from primed animals (Reiner and Locksley, 1995) and are found in patients suffering from autoimmune or allergic diseases (Romagnani, 1994), they 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. 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 (Openshaw et al., 1995). Differentiated $CD4^+$ cells characterized by unique cytokine secretion, such as Tr1 cells, have also been described. Tr1 cells produce high levels of IL-10, low levels of IL-2 and IFN-γ and no IL-4, and are able to suppress antigen-specific T cell responses (Groux et al., 1997). Therefore, the diversity of T cell subsets cannot be easily compressed into the Th1/Th2 paradigm and there are clearly more patterns of cytokine secretion than expected from a straightforward polarization in Th1 and Th2 phenotypes (Mosmann and Sad, 1996).

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 of Th1 cells may be related to the lack of IFN-γR β chain expression in Th1 cells (Pernis et al., 1995). 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 (Bach et al., 1995). Conversely, developmental commitment to the Th2 lineage results from rapid loss of IL-12 signaling in Th2 cells (Szabo et al., 1995). The inability of Th2 cells to respond to IL-12 appears to be due to selective down-regulation of IL-12R β2 subunit (Rogge et al., 1997; Szabo et al., 1997). Inhibition of Th1 and induction of Th2 in vivo is also related to down-regulation of the IL-12R β2 subunit expression (Galbiati et al., 1998). These findings are therefore consistent with a general model in which selective modulation of IL-12 signaling plays an important role in the acquisition of polarized Th cell phenotypes.

Th1 and Th2 cells in IDDM

The Th1/Th2 paradigm, originally applied to parasitic diseases, has subsequently been extended to interpret the pathogenesis of autoimmune diseases, in which the relative role of Th1 and Th2 cells is currently being actively explored. At present, the results indicate a pathogenic role of Th1 cells in many organ-specific autoimmune diseases (O'Garra and Murphy, 1993; Powrie and Coffmann, 1993; Liblau et al., 1995; Trembleau et al., 1995), whereas the role of Th2 cells is still unclear. Here we will only consider insulin-dependent diabetes mellitus (IDDM), but a similar reasoning could be applied to other experimental autoimmune diseases, such as experimental allergic encephalomyelitis (EAE), collagen-induced arthritis (CIA), experimental colitis and uveoretinitis.

Evidence for the involvement of Th1 cells in IDDM is based on adoptive transfer experiments demonstrating that $CD4^+$ cells producing Th1-type lymphokines can transfer IDDM to non-obese diabetic (NOD) mice (Bergman and Haskins, 1994; Katz et al., 1995). However, in some cases, β cell destruction in NOD mice has been associated with Th2 rather than Th1 cells (Anderson et al., 1993; Akhtar

et al., 1995). This may reflect a dual role of cytokines in disease, for example TNF-α can induce or protect from IDDM depending on the developmental stage of the immune system (Yang et al., 1994). TNF-α participates in disease development once the process has been initiated, but it appears to prevent it, by inducing tolerance to pancreatic antigens, if present before disease onset (McSorley et al., 1997).

The reciprocal regulation between T cell subsets predicts a role for Th2 cells in the inhibition of IDDM development. Evidence for a protective role of Th2 cells is provided by the reduced IDDM incidence following IL-4 (Cameron et al., 1997) or IL-10 (Pennline et al., 1994) administration to NOD mice. However, at variance with the latter result, transgenic expression of IL-10 accelerates IDDM, possibly because B cells stimulated by IL-10 would activate T cells specific for cryptic determinants of self antigens (Lee et al., 1996). 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 (Fowell and Mason, 1993) and the correlation between protection from IDDM and IL-4 production in double-transgenic mice on BALB/c background (Scott et al., 1994). In contrast to their benign role in normal NOD mice, Th2 cells have been shown to induce acute pancreatitis and IDDM in NOD-scid recipients, via production of IL-10 but not IL-4 (Pakala et al., 1997). This suggests that lymphocyte-deficient recipients lack T cells which regulate Th2 responses in normal mice. NOD mice that express IL-4 in their pancreatic β cells are protected from insulitis and IDDM (Mueller et al., 1996). Protection in NOD-IL-4 mice appears to be mediated by the pancreatic tissue itself, which causes the activation of distinct, non pathogenic T cell specificities. Regulatory T cells are not induced, as shown by the failure of spleen cells from NOD-IL-4 mice to inhibit IDDM transfer by diabetogenic T cells. These results are consistent with the observation that 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 did they provide substantial protection (Katz et al., 1995). Similar results were also obtained by adoptive transfer of non transgenic Th1 and Th2 cell lines (Healey et al., 1995). Therefore, these data do not support the concept that Th2 cells afford protection from IDDM, at least in the effector phase of the disease.

Collectively, these results point to a pathogenic role of Th1 cells in the induction of IDDM, whereas the influence of Th2 cells is still controversial. Although Th2 cells fail to inhibit effector Th1 cells in co-transfer experiments (Healey et al., 1995; Katz et al., 1995), a different view, more in line with the basic tenets of the Th1/Th2 paradigm, is that Th2 responses can be functionally dominant and actively inhibit pathogenic Th1 activity (Tian et al., 1997). In line with this concept, a relative lack of IL-4 production by NOD $CD4^+$ cells has been implicated in IDDM development. Similarly, in human diabetes, the deficient IL-4 production seen at IDDM onset may play a role by allowing disease progression (Berman et al., 1996).

Does the Th1 pathogenic/Th2 protective paradigm really hold in IDDM? The available results do not allow to draw a firm conclusion. To address the relative roles of Th subsets in IDDM we have used IL-12 as a probe.

IL-12: a probe for testing the Th1/Th2 paradigm in IDDM

IL-12 is a heterodimer composed of two covalently linked glycosylated chains, p35 and p40, encoded by distinct genes. This cytokine, produced predominantly by activated monocytes and dendritic cells enhances proliferation and cytolytic activity of NK and T cells, and stimulates their IFN-γ production. Most importantly, IL-12 induces the development of Th1 cells in vitro and in vivo. In addition, IL-12 is a potent cofactor stimulating growth, IFN-γ synthesis, and cell adhesion of already differentiated Th1 cells (Gately et al., 1998). The key role of IL-12 in the induction of Th1 cell-mediated autoimmune diseases is clearly documented in several experimental models (Adorini et al., 1997). We have used IL-12 and IL-12 antagonists, as well as NOD mice deficient in IL-12 or IFN-γ, to test the functional relevance of Th1 and Th2 cells in IDDM and to assess the mechanistic value of the Th1/Th2 paradigm in the pathogenesis of diabetes.

Exogenous IL-12

Administration of IL-12 induces rapid onset of IDDM in 100% of NOD female mice, whereas only about 70-80% of control littermates eventually develop IDDM (Trembleau et al., 1995). This effect is not due to toxicity of IL-12 for pancreatic β cell, as shown by the normal appearance of islet cells and 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. Intracytoplasmic staining for IFN-γ and IL-4 production demonstrates that pancreas-infiltrating $CD4^+$ cells in adult NOD mice are exclusively of the Th1 type, and they are increased by 3 fold following IL-12 administration. These results are therefore consistent with a dominant role of Th1 cells in the pathogenesis of IDDM.

Conversely, following a protocol developed by O'Hara and coworkers (O'Hara et al., 1996), we could confirm that intermittent administration of IL-12 (once weekly for 12 weeks) to NOD mice delays and reduces IDDM development. Data explaining these opposite effects of IL-12 are not yet available. However, it is conceivable that intermittent administration of IL-12, while still favoring Th1 induction, may be unable to sustain Th1-cell development. Thus, the aborted induction of a Th1 response, possibly coupled with the emergence of regulatory Th2 cells, may result in the delay of Th1-mediated disease progression. This result should not be considered a surprising paradox, but rather an expected property of Th1/Th2 cell regulation.

Endogenous IL-12

IL-12 may thus have a primary role in IDDM induction, rendering IL-12 antagonists attractive candidates for immunointervention. A natural antagonist of IL-12 is the IL-12 p40 molecule itself. The mouse p40 chain specifically antagonizes mouse IL-12 p75 and the primary inhibitory species is a disulfide-linked homodimeric form of IL-12 p40, termed $(p40)_2$. Based on competitive binding assays performed under

high affinity binding conditions, mouse $(p40)_2$ appears to bind to the mouse IL-12R with an affinity similar to that of IL-12, but it does not trigger biologic activity and specifically inhibits IL-12-mediated responses (Gately et al., 1998).

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 $(p40)_2$ (Trembleau et al., 1997). 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 as well as 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 (Rothe et al., 1997), 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.

Collectively, these results indicate that the extent of immune deviation to Th2 is related to the degree of protection from IDDM, as predicted from the Th1/Th2 paradigm. However, they do not analyze whether Th2 cells are directly responsible for protection from IDDM or whether the immune deviation away from Th1 in itself accounts for the decreased IDDM incidence.

IDDM in IL-12- or IFN-γ-deficient NOD mice

To further 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 (Magram et al., 1996) and backcrossed to the NOD background. Antigen priming in $IL\text{-}12^{-/-}$ NOD mice gives rise to antigen-specific T cells that are able to secrete IL-2, IL-4, IL-5 and IL-10 in amounts comparable to littermate controls, but show a three-fold reduction in IFN-γ secretion.

IDDM develops in $IL\text{-}12^{-/-}$ NOD mice with a similar incidence as that in controls, indicating that IL-12 is dispensable (S. Trembleau et al., manuscript in preparation). Therefore, targeting endogenous IL-12 does prevent IDDM (Trembleau et al., 1997), 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 (Rothe et al., 1997). However, it remains to be determined whether IL-18 can induce the development of Th1 cells in IL-12-deficient NOD mice.

A similar situation has been described for IFN-γ itself, a cytokine produced by Th1 cells which as been implicated in the effector mechanisms leading to β cell destruction. Inhibition of endogenous IFN-γ protects from disease (Campbell et al., 1991; Debray-Sachs et al., 1991), but IDDM develops in IFN-γ-deficient NOD mice (Hultgren et al., 1996). However, insulitis does not develop in IFN-γR-deficient NOD mice (Wang et al., 1997). While there is no clear explanation at present for the discrepancy between IFN-γ and IFN-γR-deficient mice, it should be noted that it has been observed also in other models (Reiner and Locksley, 1995). In addition, development of insulitis and IDDM does not occur in IFN-γ-deficient RIP-LCMV-transgenic mice (von Herrath and Oldstone, 1997), admittedly a more artificial system in which to study autoimmune diabetes.

Overall, these conflicting results may 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, in accord with what already noted for EAE (Steinman, 1997). 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 (Magram et al., 1996) 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.

Conclusions

Based on our current results, we would draw the following conclusions:

1. IDDM in NOD mice is associated with pancreatic infiltration of Th1-type cells. Acceleration of IDDM by IL-12 administration is associated with enhanced infiltration of Th1 cells. Both results are consistent with a pathogenic role of Th1 cells in IDDM.
2. Deviation of pancreas-infiltrating cells to the Th2 phenotype by targeting endogenous IL-12 is associated with protection from IDDM. The degree of immune deviation is related to the level of protection. These data indicate that Th2 cells can enter pancreatic islets but are non pathogenic in NOD mice. Whether protection only reflects immune deviation away from Th1 or it involves a direct protective effect of Th2 cells remains to be established.
3. IL-12 is dispensable for IDDM development. At face value, the Th1/pathogenic side of the paradigm could hold only if pathogenic Th1-type cells would develop in the absence of IL-12. Some Th1 cell development has been shown to occur in the absence of IL-12 (Magram et al., 1996) and we are presently evaluating the extent of Th1-type cell development in IL-12-deficient NOD mice.

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