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Cutting Edge: Interactions Through the IL-10 Receptor Regulate Autoimmune Diabetes

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BDC2.5/nonobese diabetic (NOD) transgenic mice express a TCR from a diabetogenic T cell clone yet do not spontaneously develop diabetes at high incidence. Evidence exists showing that in the absence of endogenous TCR α-chain rearrangements this transgenic mouse spontaneously develops diabetes and that CTLA-4 negatively regulates diabetes onset. This strongly suggests that onset of diabetes in BDC2.5/NOD mice is governed by T cell regulation. We addressed the mechanism of immune regulation in BDC2.5/NOD mice. We find that activated spleen cells from young, but not old, BDC2.5/NOD mice are able to transfer diabetes to NOD-scid recipients. We have used anti-IL-10R to show that the failure of splenocytes from older mice to transfer diabetes is due to dominant regulation. We furthermore found that diabetes developed following anti-IL-10R treatment of 6-wk old BDC2.5/NOD mice indicating that endogenous IL-10 plays a key role in the regulation of diabetes onset in this transgenic mouse. The Journal of Immunology, 2001, 167: 6087–6091.

For many years it has been suggested that autoimmune disease may result in part from a deficit in regulatory T cells (1). Considerable evidence has been amassed recently to support that hypothesis. Several groups have shown that autoimmunity may develop following induction of lymphopenia (2–4) and the resultant pathology can be prevented by restoration of a subpopulation of CD4+ T cells (5–8). The identification of this subpopulation, which is able to modulate the development of autoimmune pathology, has led to the proposal that autoimmune disease may result from some deficit in these regulatory cells. A variety of markers have been used to define these CD4+ cells which, in the mouse, include CTLA-4 and CD25 (9).

The nonobese diabetic (NOD) mouse provides an excellent model of human insulin-dependent diabetes mellitus (IDDM) (10). In this mouse model, insulinitis develops from 4–5 wk of age and mice develop diabetes from around 12 wk of age. The BDC2.5 TCR-transgenic mouse (11) expresses the rearranged TCR α- and β-chain genes (Vα1Vβ4) of a diabetogenic CD4+ T cell clone, BDC2.5 (12). When the BDC2.5 NOD TCR-transgenic line is made homozygous for scid, Cα- , or RAG- , the mice spontaneously develop IDDM at around 3–5 wk of age, whereas on a RAG+ background the incidence is very low (13, 14). This has led to the suggestion that T cells expressing endogenously rearranged TCR α genes might be playing a role in the prevention of diabetes in the BDC2.5 TCR-transgenic mouse as has been proposed for experimental autoimmune encephalomyelitis in myelin basic protein-specific TCR-transgenic mice (15). We have conducted experiments to determine whether there is any evidence in the BDC2.5 TCR-transgenic mouse for active suppression of diabetogenic T cells. It had previously been shown that although this transgenic mouse does not itself develop diabetes at high incidence (and then mostly at an advanced age), it was possible to transfer disease reproducibly into NOD-scid recipients using Con A-activated spleen cells from the BDC2.5 transgenic mouse (14). This shows that potentially diabetogenic effector cells must be present in the nondiabetic BDC2.5 transgenic mouse spleen. The possibility that such potentially diabetogenic T cells are held in check by regulatory cells has been inferred from studies showing that anti-CTLA-4 Abs can induce IDDM in very young BDC2.5 TCR-transgenic mice (16). We have used the Con A activation and transfer system to identify mechanisms contributing to regulation of these diabetogenic T cells. We found that disease transfer reproducibly occurred only when donor cells were taken from young mice and that the ability to transfer disease diminished with the age of the mouse. Our studies show diabetogenic cells remain in the spleen cell pool derived from older mice, as treatment of NOD-scid recipients with Ab to the IL-10R permitted disease transfer by these cells. A key role for the IL-10R in modulating IDDM was further shown by the ability of IL-10R-specific Ab to bypass the requirement for Con A activation of donor splenocytes for disease transfer and furthermore directly promote the onset of IDDM in BDC2.5 TCR-transgenic mice.

Materials and Methods

Mice

NOD, NOD-scid, BDC2.5/NOD, and BDC2.5/NOD-scid transgenic mice were maintained in the Biological Services facility of the Department of Pathology (University of Cambridge, Cambridge, U.K.). NOD-scid mice or transgenic mice crossed on to the NOD-scid background were maintained in microisolator cages with filtered air.

Abs and reagents

The hybridomas 1B1.3a (anti-IL-10R) and GL113 (isotype control) (rat IgG1) were obtained from Dr. K. Moore (DNAX, Palo Alto, CA). Ab was harvested from culture supernatants by ammonium sulfate precipitation.
reagents were obtained from Sigma-Aldrich (Poole, Dorset, U.K.). Other Abs were obtained from BD PharMingen (Oxford, U.K.) and reagents were obtained from Sigma-Aldrich (Poole, Dorset, U.K.).

**In vitro culture of splenocytes**

Single cell suspensions of BDC2.5/NOD mouse spleens were cultured under standard conditions for varying times up to 72 h in the presence of Con A (5 μg/ml) or islets. For cytokine ELISAs and FACS staining anti-IL-10R or isotype control Ab preparations were added (30 μg/ml). For transfer into recipient mice, residual Con A was removed by addition of methyl-a-D-mannopyranoside (10 mg/ml), and the cells were washed three times.

**CD25+ cell depletion**

Splenocytes were depleted of CD25+ cells by incubating with biotinylated anti-CD25 Ab (BD PharMingen) followed by streptavidin Dynabeads (Dynal Biotech, Wirral, U.K.) and passage over a magnet (>2).

**Islet isolation**

Male NOD mice were sacrificed and the common bile duct was infused in situ with collagenase. The pancreas was then removed and digested at 37°C. The islets were enriched on a Eurocollins-Ficoll gradient and washed in HBSS containing BSA, and islets were handpicked using a siliconized micropipette.

**ELISA for IL-10, IL-12 and IFN-γ**

Sera or supernatants were assessed for the presence of cytokines using a capture ELISA (Optea anti-mouse cytokine kits; BD PharMingen). The concentration of cytokine was calculated from graphs for IL-10, IL-12 and IFN-γ standard preparations.

**Flow cytometric analysis**

Cells were stained by standard methods using rat anti-mouse reagents CD4-FITC, CD25-PE, and CD69-PE (BD PharMingen) and analyzed on a BD Biosciences FACScan instrument (BD Biosciences, Oxford, U.K.).

**In vivo Ab treatment**

In the transfer experiments recipients were treated with 0.5 mg of the Ab preparation (or isotype control) i.p. on days 0, 1, 4, and 7. BDC2.5/NOD mice were given the same dose i.p. on days 0, 5, and 14.

**Assessment of diabetes**

Recipient mice were tested for the presence of urinary glucose using Diastix (Bayer, Newbury, U.K.). Blood glucose was measured using an Esprit glucometer and glucose test strips (Bayer). Mice were considered diabetic if they had urinary glucose and blood glucose concentrations of 12 mM or above on two occasions.

**Histological examination**

Pancreases were processed for wax histology. Five-micrometer sections were taken at eight levels (200 μm apart) and stained with H&E. Total islets per section were counted and the degree of cellular infiltration was scored.

**Results and Discussion**

The transfer of type 1 diabetes by activated spleen cells from BDC2.5 TCR-transgenic NOD mice diminishes with age

Female NOD mice usually develop IDDM from 12 wk of age, achieving an incidence of around 80% at 30 wk of age. However, diabetes does not develop in BDC2.5 TCR-transgenic mice at such high incidence (13). This is not due to an absence of potentially diabetogenic effector cells, because Con A-activated splenocytes from 6-wk old BDC2.5 TCR-transgenic mice are able to transfer diabetes to NOD-scid recipients (Ref. 14 and Fig. 1A). We find that this ability to transfer IDDM decreases with the age of the donor mouse. Diabetes is transferred within 14 days by splenocytes from donor mice of less than 10 wk of age, whereas disease transfer is less reliable when activated splenocytes from older donors are used and the time to disease onset is extended (Fig. 1A). The impaired ability of splenocytes from older mice to transfer disease is not due to their failure to traffic to the islets, as splenocytes from older BDC2.5/NOD mice caused a significant degree of insulitis in NOD-scid recipients (Fig. 1, B and C).

CD25+ cells are not responsible for the lack of disease transfer by splenocytes from older BDC2.5/NOD mice

As it was possible that there was an increased ratio of regulatory to effector cells in the spleens of older mice, we looked for evidence for regulatory cell-mediated inhibition of disease transfer. It has been shown that CD4+CD25+ T cells are able to down-regulate autoreactive or inflammatory responses (17–21). CD4+CD25+ T cells are present only in very low numbers (0.9%) in spleens of BDC2.5 NOD-scid mice, whereas they are clearly present in the spleens of NOD and BDC2.5 TCR-transgenic mice (10.2% and 5.6% respectively). All BDC2.5 NOD-scid mice develop disease when 3–5 wk old, whereas BDC2.5 TCR NOD transgenic mice do not develop diabetes at a high incidence. To determine whether the presence of CD4+CD25+ T cells contributed to this low incidence of diabetes we depleted CD25+ cells from the splenocyte pools of older transgenic mice before Con A activation and examined their ability to transfer disease. Depletion of CD25+ cells failed to enable diabetes transfer by activated splenocytes from older mice (zero of four mice in each of the depleted and control groups). Comparable levels of insulitis developed in NOD-scid recipients of both depleted and nondepleted spleen cells (Fig. 2A). Therefore, there is no evidence for CD25+ T cell-mediated prevention of diabetes transfer in this experimental system. This result parallels the observation in the rat where peripheral CD4+ T cells, which prevent diabetes, are found in both CD25+ and CD25− populations (22).
Ab specific for IL-10R promotes onset of diabetes

Because IL-10 has been implicated in regulation of inflammation and autoimmunity (23), we examined whether Ab which is specific for, and blocks, the IL-10R (24) influenced the ability of activated splenocytes from older mice to transfer diabetes. Fig. 2B shows that these are able to cause diabetes in anti-IL-10R-treated NOD-scid recipients. These data suggest that interactions through the IL-10R play a key role in the regulation of type 1 diabetes in BDC2.5/NOD mice.

It was not possible to transfer IDDM with nonactivated splenocytes from young BDC2.5 TCR-transgenic mice (14). To determine whether this failure to transfer disease also involved interactions through the IL-10R we transferred splenocytes from young BDC 2.5 TCR-transgenic mice without Con A activation into NOD-scid recipients in the presence or absence of anti-IL-10R. Treatment of recipients with anti-IL-10R Ab led to the development of IDDM (seven of nine compared with zero of nine control mice). This was also seen when spleen cells from older BDC2.5 TCR-transgenic mice were used (eight of eight compared with zero of eight control mice). To determine whether the anti-IL-10R Ab had caused direct T cell activation, spleen cells were cultured with IL-10R Ab and analyzed by FACS for expression of CD25 and CD69. There was no evidence that anti-IL-10R caused T cell activation (Fig. 2C). A further indication that anti-IL-10R did not directly activate diabetogenic effector cells is provided by our observation that BDC 2.5 NOD-scid mice treated with control or anti-IL-10R Ab develop type 1 diabetes with comparable kinetics (data not shown).

The ability of IL-10R Ab to promote disease in these transfer experiments suggested that such cytokine-mediated control might account for the low incidence of diabetes in BDC2.5 TCR-transgenic mice. BDC2.5/NOD mice (6 wk) were given three injections of either anti-IL-10R or control Ab and monitored for disease onset. The anti-IL-10R Ab-treated mice started to develop diabetes at 11 wk of age, and by 23 wk 100% were diabetic. None of the control mice developed diabetes and remained disease free for >30 wk (Fig. 3A). These data strongly suggest that in vivo an IL-10-like cytokine plays a regulatory role in preventing diabetes development in BDC 2.5 TCR-transgenic mice.

Does anti-IL-10R Ab influence cytokine production?

As early as 24 h following one injection of anti-IL-10R, serum levels of IL-12 were elevated. This increased IL-12 was sustained over the assessment period of 13 days (Fig. 3B). The presence of elevated serum IL-12 suggested that IL-10R Ab treatment was favoring a proinflammatory response. Therefore, we assessed whether anti-IL-10R could influence the production of proinflammatory cytokines in vitro. BDC2.5/NOD splenocytes were cultured for 48 h with Con A in the presence of anti-IL-10R or control anti-IL-10R or GL113. The solid and dotted lines represent the GL113 and anti-IL-10R treated populations, respectively, while the gray line shows that Con A treatment increased expression of the activation markers CD69 and CD25.
Ab. The production of both IL-12 and IFN-γ was increased when the TCR-transgenic splenocytes were cultured in the presence of Con A and anti-IL-10R in vitro (Fig. 3C). Comparable experiments were conducted using isolated NOD islets to stimulate T cell responses. As shown in Fig. 3D, there was an increased production of IL-12 and IFN-γ when BDC2.5/NOD spleen cells were cultured in the presence of an anti-IL-10R compared with an isotype control Ab. These data strongly suggest that anti-IL-10R facilitates the onset of diabetes by permitting the expression of a Th1 response against islets and that the development of diabetes in BDC2.5/NOD mice is endogenously regulated by interactions through this receptor.

A regulatory population of T cells which both produces and responds to IL-10 has been identified in both humans and mice (8, 25). T cells are clearly involved in the regulatory process that prevents diabetes in BDC2.5/NOD mice. It is possible that these cells use a TCR with an endogenously rearranged α-chain, because they are not present in BDC2.5/NOD−/− mice. By producing IL-10 such regulatory T cells could influence the development of type 1 diabetes in many ways. Th1 cells, dendritic cells (DC), and macrophages play a role in the development of diabetes (26). IL-10 is known to be able to inhibit both DC and macrophage function. IL-10 has been shown to be an autocrine growth factor for DC, and neutralization of IL-10, together with a DC maturation signal, has been shown to increase expression of CD80, CD86, and MHC, as well as production of IL-12 and TNF-α. Anti-IL-10 treatment of BDC2.5/NOD mice resulted in elevated serum IL-12 levels (Fig. 3B) while its inclusion in vitro clearly enhanced both IL-12 and IFN-γ production in response not only to Con A but also to islets (Fig. 3, C and D). Prevention of an IL-10/IL-10R interaction in BDC2.5/NOD mice would therefore be predicted to skew the T cell response to Th1 and thus be potentially diabeticogenic. IL-10 inhibits production of a range of inflammatory mediators, including IL-1α, IL-1β, and TNF-α, by macrophages (27, 28). These cytokines, together with IFN-γ, have been shown to be particularly damaging to pancreatic β cells (29). Inhibition of macrophage function would therefore be expected to provide additional protection from autoimmune destruction.

There are several studies showing that administration of exogenous IL-10 (as IL-10 itself, an IL-10/Fc fusion protein, or a viral homolog of IL-10) is able to prevent development of insulitis or diabetes onset in NOD mice (30–32). Furthermore, transfer of anti-IL-10R such regulatory T cells could in consequence of developmental abnormality of a T cell subpopulation. 3. Nishizuka, Y., T. Sakakura, and A. Kojima. 1973. Murine thyroiditis induced by neonatal thymectomy. Exp. Immunol. 19:396.


