T Helper 2 (Th2) T Cells Induce Acute Pancreatitis and Diabetes in Immune-compromised Nonobese Diabetic (NOD) Mice

By Syamasundar V. Pakala, Michael O. Kurrer, and Jonathan D. Katz

From the Department of Pathology and Center for Immunology, Washington University School of Medicine, St. Louis, Missouri 63110

Summary

Autoimmune diabetes is caused by the $CD4^+$, T helper 1 (Th1) cell-mediated apoptosis of insulin-producing β cells. We have previously shown that Th2 T cells bearing the same T cell receptor (TCR) as the diabetogenic Th1 T cells invade islets in neonatal nonobese diabetic (NOD) mice but fail to cause disease. Moreover, when mixed in excess and cotransferred with Th1 T cells, Th2 T cells could not protect NOD neonates from Th1-mediated diabetes. We have now found, to our great surprise, the same Th2 T cells that produced a harmless insulitis in neonatal NOD mice produced intense and generalized pancreatitis and insulitis associated with islet cell necrosis, abscess formation, and subsequent diabetes when transferred into immunocompromised NOD.*scid* mice. These lesions resembled allergic inflamation and contained a large eosinophilic infiltrate. Moreover, the Th2-mediated destruction of islet cells was mediated by local interleukin-10 (IL-10) production but not by IL-4. These findings indicate that under certain conditions Th2 T cells may not produce a benign or protective insulitis but rather acute pathology and disease. Additionally, these results lead us to question the feasibility of Th2-based therapy in type I diabetes, especially in immunosuppressed recipients of islet cell transplants.

Insulin-dependent diabetes mellitus (IDDM)[1] is caused by the autoimmune destruction of insulin-producing β cells in the islets of Langerhans of the pancreas (1, 2). The leukocytic infiltration, termed insulitis, is a heterogeneous mixture of $CD4^+$ and $CD8^+$ T lymphocytes, B lymphocytes, macrophage and dendritic cells (3). In general, T lymphocytes, play the most pivotal role in initiating the disease process (4–7). Recently, we demonstrated that islet cell antigen-responsive $CD4^+$ T cells alone are sufficient for the spontaneous development and transfer of diabetes in NOD.*scid* mice (8).

$CD4^+$ T cells can be differentiated into at least two major subsets, Th1 cells that secrete IFN-γ and Th2 cells that produce IL-4 and IL-10. Th1 cells are critically involved in the generation of effective cellular immunity (9), whereas Th2 T cells are instrumental in the generation of humoral and mucosal immunity and allergy, including the activation of eosinophils and mast cells and the production of IgE (9). A number of studies have now correlated diabetes with Th1 phenotype development (10–12). On the other hand, Th2 T cells were shown to be relatively innocuous (12–15). Some have even speculated that Th2 T cells in fact, may be protective (16–19), although direct evidence in support of Th2-mediated protection is lacking.

We have previously reported that the ability of $CD4^+$ T cells to transfer diabetes to naïve recipients resided not with the antigen specificity recognized by the TCR, per se, but with the phenotypic nature of the T cell response (12). Strongly polarized Th1 T cells transferred disease into NOD neonatal mice, while Th2 T cells did not, despite being activated and bearing the same TCR as the diabetogenic Th1 T cell population. Moreover, upon cotransfer, Th2 T cells could not ameliorate the Th1-induced diabetes, even when Th2 cells were cotransferred in ~10-fold excess (12).

However, we wondered if we transferred Th2 cells before transfer of Th1 cells, we might amplify the protective influence of Th2 cells, and thereby control the subsequent Th1 T cell response. In this study, we attempt to evaluate directly this hypothesis by producing Th1 and Th2 T cells from the islet-reactive BDC2.5 TCR transgenic NOD mouse (20), and then transfer sequentially Th2 and then Th1 T cells into NOD recipients mice.

Much to our surprise, we found that Th2 T cells exerted markedly differing effects on NOD recipients depending on the immune status of the recipient mouse. Here, we report that while Th1-polarized T cells can transfer disease in neonatal NOD mice, something Th2-polarized T cell fail

[1] *Abbreviations used in this paper:* AEC, aminoethylcarbazole; DAB, diaminobenzidine; IDDM, insulin-dependent diabetes mellitus; PNA, peripheral node addressin.

to do, both Th1- and Th2-polarized T cells can transfer disease in NOD.*scid* mice and other immune-compromised recipients. The Th2-mediated diabetes in NOD.*scid* recipients exhibited a longer prediabetic phase and a lowered overall incidence. Moreover, the diabetic lesion created by Th2 cells was unique and quite unlike the lesion found in spontaneously diabetic BDC2.5 mice or Th1 T cell–induced diabetes in either neonates or NOD.*scid* mice. That Th2 cells caused a distinct but important disease in immune-compromised recipients has significant implications with regard to the potential use of Th2 cells as a therapeutic agent.

Materials and Methods

Mice. BDC2.5 TCR transgenic mice were described previously (20). Mice used in these experiments were housed under specific pathogen-free conditions and were backcrossed to NOD/lt for >20 generations. The NOD.*scid* mice were bred under pathogen-free conditions at Washington University from an original breeding stock provided by Dr. E. Leiter of the Jackson Laboratories.

Flow Cytometry. Flow cytometry was performed on either a Becton Dickinson FACScan® or FACSVantage® flow cytometer. We purchased anti-CD4–PE mAb (Caltag Laboratory, South San Francisco, CA). mAb to the β chain of the transgenic TCR, KT4-10 (21), was purified from ascites and conjugated with FITC. List mode data was collected on 1×10^5 cells and reanalyzed using WinMDI (version 2.1.4) software written by J. Trotter (http://facs.scripps.edu).

Diabetes. Diabetes was assessed by measurement of venous blood using a Bayer Glucometer Elite one-step blood glucose meter. Animals were considered diabetic after two consecutive measurements ⩾250 mg/dl (13.75 mM). Onset of diabetes was dated from the first consecutive reading. In most instances, sustained hyperglycemia of >500 mg/dl was observed, and animals were killed to avoid prolonged discomfort.

Immunohistochemistry. Mice were killed by cervical dislocation or CO_2 asphyxiation. The entire pancreata were removed, fixed either in 10% neutral-buffered formalin at 4°C for at least 20 h but not more than 26 h, embedded in paraffin, and sections (2 μm), collected on poly l-lysine coated slides (VWR Scientific Products, Corp., Philadelphia, PA). Alternatively, pancreata were snap frozen in OCT compound (Tissue-tek) for cryosectioning. 5-μm cryosections were obtained, air dried, and stored at −20°C until used. Formalin-fixed sections were deparaffinized in xylene and alcohol, and stained with hematoxylin and eosin for general morphology.

Sections were stained using the TUNEL procedure (modified from reference 22) to detect apoptosis as described previously (8) and counterstained with hematoxylin. In brief, sections were digested with Proteinase K (20 μg/ml for 15 min), endogenous peroxidase was blocked in hydrogen peroxide (3% for 5 min), and residual avidin or biotin was quenched by sequential incubation in avidin (20 μg/ml for 20 min) or biotin (10 μg/ml for 20 min). Sections were then preincubated in 1.5 mM $CoCl_2$ TdT buffer (Boehringer Mannheim, Indianapolis, IN). Terminal deoxynucleotide transferase (TdT, Boehringer Mannheim) was then used to label nicked and fragmented DNA with biotin-conjugated dUTP at 37°C for 100 min (1.5 mmol $CoCl_2$, 0.6 μl TDT, 1.5 μl biotin–dUTP per 100 μl buffer ~30 μl/4 cm^2). Slides were washed in 2× SSC (15 min), incubated in 2% BSA (ELISA grade, Sigma, St. Louis, MO) followed by Streptavidin–horseradish peroxidase (Caltag, 1:1000, 30 min at 37°C). The slides were developed with diaminobenzidine (DAB; Pierce, Rockford, IL) for 2 min.

Immunohistochemistry for insulin was then performed using a two-step protocol. Endogenous peroxidase activity was blocked, and slides were incubated with rabbit antiserum to insulin (Dako Corp., Carpenteria, CA; 1:500 in 5% normal mouse serum for 30 min). After wash steps, staining was revealed with horseradish peroxidase–conjugated anti-rabbit Ig (Dako; 1:500 in 5% NMS for 30 min), developed with aminoethylcarbazole (AEC; Pierce) for 10 min and mounted in crystalmount.

Cryosections were allowed to equilibrate to room temperature and rehydrated in PBS for 10 min. Endogenous peroxidase activity was blocked, and slides were incubated with antibodies against Vβ4 TCR (KT4) and CD4, macrophage subpopulations (MAC-1, MOMA-1, MOMA-2, ERMP-23; all gifts of Dr. P. Leenen, Erasmus University, Rotterdam, The Netherlands), dendritic cells (NLDC-145; Harlan Sera Labs, Indianapolis, IN), natural killer cells (DX5; a gift of Dr. L. Lainer, DNAX, Palo Alto, CA), mad-CAM (MECA-367), and peripheral node addressin (MECA-79; both mAb gifts from Dr. P.R. Streeter, Monsanto, St. Louis, MO) for 60 min at room temperature in a humidified container. After wash steps, staining was revealed with horseradish peroxidase–conjugated goat anti–rat Ig (Caltag; 1:500 in 5% NMS for 30 min), developed with AEC for 10 min, and mounted in crystalmount. For each pancreas, multiple parallel sections were analyzed in five levels, each 0.5 mm apart, in order to minimize sample error.

T Cell Transfers. Naive $CD4^+$ T cells were enriched from thymi of BDC2.5/NOD mice by depleting $CD4^+$ $CD8^+$ double-positive cells with anti-CD8 antibody and rabbit complement (Cederlane, Ontario, Canada). T cells were resuspended at 1×10^5 cells/ml in DMEM supplemented with 10% heat-inactivated fetal bovine serum, 1 mM sodium pyruvate, 1 mM glutamine, and 50 μM 2-mercaptoethanol and incubated with 2×10^6 APC/ml and 2×10^4 islet cells in conditions previously demonstrated to generate Th1 or Th2 cells for 7 d (12, 23). In brief, T cells were cultured in the presence of islet cells and NOD splenocytes in the presence of IFN-γ (50 U/ml) and IL-12 (50 U/ml), and neutralizing anti–IL-4 antibodies (11B11, 10 μg/ml) for generation of Th1 T cells or IL-4 (50 U/ml) and neutralizing amounts of anti IFN-γ (H22, 10 μg/ml) and anti IL-12 (Tosh, 10 μg/ml) antibodies for Th2 T cells. T cells were recovered from these cultures and transferred into neonatal NOD or 6-wk-old NOD.*scid* mice. An aliquot of cells was restimulated with antigen and APC alone and supernatants were checked for the presence of appropriate cytokines by ELISA to confirm polarization of T cells. Neonatal NOD mice were injected with 1×10^6 $CD4^+$ $V\beta4^+$ Th1 or Th2 T cells intraperitoneally, whereas NOD.*scid* and TCR-αβ–deficient NOD (NOD.$C\alpha^{-/-}$) mice were injected with 1×10^6 cells intravenously.

In mice treated with neutralizing cytokine antibodies, mice received 250 μg of anti-IL-4 (11B11), or anti-IL-10 (2A5) 1 d before receiving Th2 cells and then every 3 d thereafter.

In Vitro Restimulation. Th2 T cells stimulated as above were recovered and cultured for an additional 7 d in the presence of antigen under either Th2 conditions (anti–IL-12, anti-IFN-γ, and IL-4), Th1 conditions (anti–IL-4, IFN-γ, and IL-12), or Th1/Th2 conditions (IL-4, IL-12, and IFN-γ) to evaluate Th2 phenotype stablity. At the end of the second 7-d cycle, recovered T cells were restimulated with islet cells and irradiated NOD APC in the absence of exogenous cytokines or mAb, and supernatant were collected and analyzed by ELISA at 24 h.

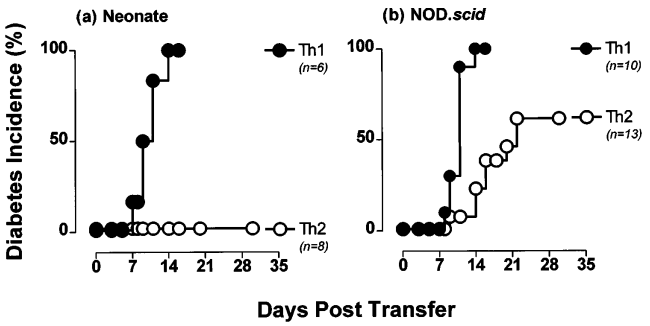

Figure 1. Transfer of diabetes with both Th1- and Th2-like cells into NOD.*scid* mice. (*a*) Th1 cells (*closed circles*) transfer diabetes into neonatal NOD mice in 7–14 d, while Th2 cells (*open circles*) do not transfer even up to 35 d. (*b*) Th1 and Th2 cells transfer diabetes into NOD.*scid* mice. Diabetes was assessed by measuring blood glucose. Mice with two consecutive blood sugar readings above 250 mg/dl were considered diabetic. Onset was dated to the first of the two consecutive readings.

Cytokine ELISA. IL-4 was detected using monoclonal anti-IL-4 antibody (11B11) as the capture antibody and revealed with rabbit anti-mouse IL-4 polyclonal serum (a gift of Dr. R.D. Schreiber, Washington University, St. Louis, MO) followed by biotin-conjugated goat anti–rabbit antibody (Vector) and Streptavidin–horseradish peroxidase (Jackson Immunoresearch, Avondale, PA) using TMB substrate (Sigma). IFN-γ was detected using H22 (gift of Dr. R.D. Schreiber) as the capture antibody and revealed with goat anti–mIFN-γ antibody followed by horseradish peroxidase–conjugated donkey anti–goat IgG (Jackson Immunoresearch) using ABTS (Sigma) as substrate.

Results

Th2 Cells Transfer Disease in NOD.scid Mice. We generated Th1 and Th2 cells from naive populations of BDC2.5/NOD.*scid* thymocytes by in vitro stimulation with islet cells in the presence of anti-IL-4, recombinant IFN-γ, and IL-12 for Th1, or anti–IL-12 and anti–IFN-γ, and recombinant IL-4 for Th2 cells. T cells taken from these cultures were restimulated in the presence of islet cells and APC alone and the supernatants were tested for the presence of appropriate signature cytokines by ELISA confirming the polarization of the T cells (data not shown). Th1- or Th2-polarized BDC2.5 T cells were then injected into either neonatal NOD (immune-competent) mice or NOD.*scid* (immune-compromised) mice, and the recipient animals were monitored for diabetes by blood glucose. Th1-injected neonatal NOD mice became diabetic by day 14, whereas Th2-injected neonatal recipient mice remained normal glycemic even after 35 d (Fig. 1 *a*). Moreover, when Th1 T cells from the same polarized cultures were transferred into 6-wk-old NOD.*scid* mice, diabetes was likewise rapidly induced (Fig. 1 *b*). The rate, progression, and penetrance of disease was identical to that seen in neonatal recipients of Th1 cells (Fig. 1, *a* and *b*), with sustained hyperglycemia appearing between day 9 and 14 after-transfer (Fig. 1 *b*). Insulitis, as determined from serial pancreas histology, started at day 5 and becoming severe by day 9 (data not shown). Moreover, when Th1 cells were transferred into NOD mice lacking αβ–T cells (NOD $C\alpha^{-/-}$), hyperglycemia resulted with similar kinetics and penetrance (data not shown). Therefore, Th1 cells produced similar disease in both immune-compromised and immune-competent mice. In addition, the Th1-mediated disease was nearly identical to the diabetes seen with BDC2.5/NOD.*scid* T cells from spontaneously diabetic animals (8).

Surprisingly, unlike the Th2 recipient neonatal NOD, which remained disease free, immune-compromised NOD mice (both NOD.*scid* and NOD $C\alpha^{-/-}$) infused with Th2 cells developed hyperglycemia (Fig. 1 *b*; data not shown). However, the kinetics and penetrance of disease were different from that seen in Th1-mediated disease. Islet-reactive Th2 cells produced disease with slower kinetics and only partial penetrance in NOD.*scid* recipients (Fig. 1 *b*). By day 16, 25% of the mice were diabetic and maximum disease penetrance was not achieved until day 24 with 60% of the mice showing sustained hyperglycemia. This slow disease seen in NOD.*scid* recipients of Th2 cells could be due to (*a*) the slow expansion of a contaminating Th1 T cell population or a switch of the input T cell population to Th1 or (*b*) a unique Th2 lesion with inherently slower kinetics.

We addressed the first possibility by taking polarized Th2 T cells and assessing their ability to switch phenotypes both in vitro and in vivo. In culture, naive BDC2.5 T cells were stimulated under conditions that generated Th2 T cells as above. However, after one round of culture, Th2 T cells were then subjected to a secondary antigenic stimulation in the presence of IL-12 and IFN-γ (Th1 condition) that favors the outgrowth of Th1 cells or the phenotypic switch of poorly polarized Th2 T cells. The presence of IFN-γ–producing cells is then revealed under neutral reconditions. Even after 1 wk of culture with IFN-γ and IL-12, we were unable to detect IFN-γ production by our Th2 cells (data not shown). More directly, splenocytes recovered from NOD.*scid* recipient mice were stimulated with plate-bound anti-CD3, and the supernatants were assayed for both Th1 and Th2 cytokines. We found that the recovered splenocytes could only secrete IL-4 in detectable quantities (data not shown). From this, we concluded that Th2 cells did not switch phenotype after polarization, nor did a contaminating Th1 population grow out from the input Th2 transfer population.

Th2 T Cells Induce a Novel Pancreatic Lesion. The best evidence supporting a unique Th2-mediated disease was radically different histopathology of the Th2 lesions in NOD.*scid* mice. Th1 T cells and those from spontaneously diabetic BDC2.5/NOD.*scid* mice produce focused islet lesions without involvement of the surrounding exocrine tissue (Fig. 2 *a*; reference 8). That is to say, the infiltrating mass of leucocytes surrounded the islets (peri-insulitis) and then proceeded to invade the islet from the periphery to the center (insulitis). The infiltrating mass was predominantly $V\beta4^+$, $CD4^+$ T cells, macrophages with a few neutrophils (see below). By contrast, Th2 T cells followed a more disorganized pattern. Leucocytes infiltrated not only the islets but

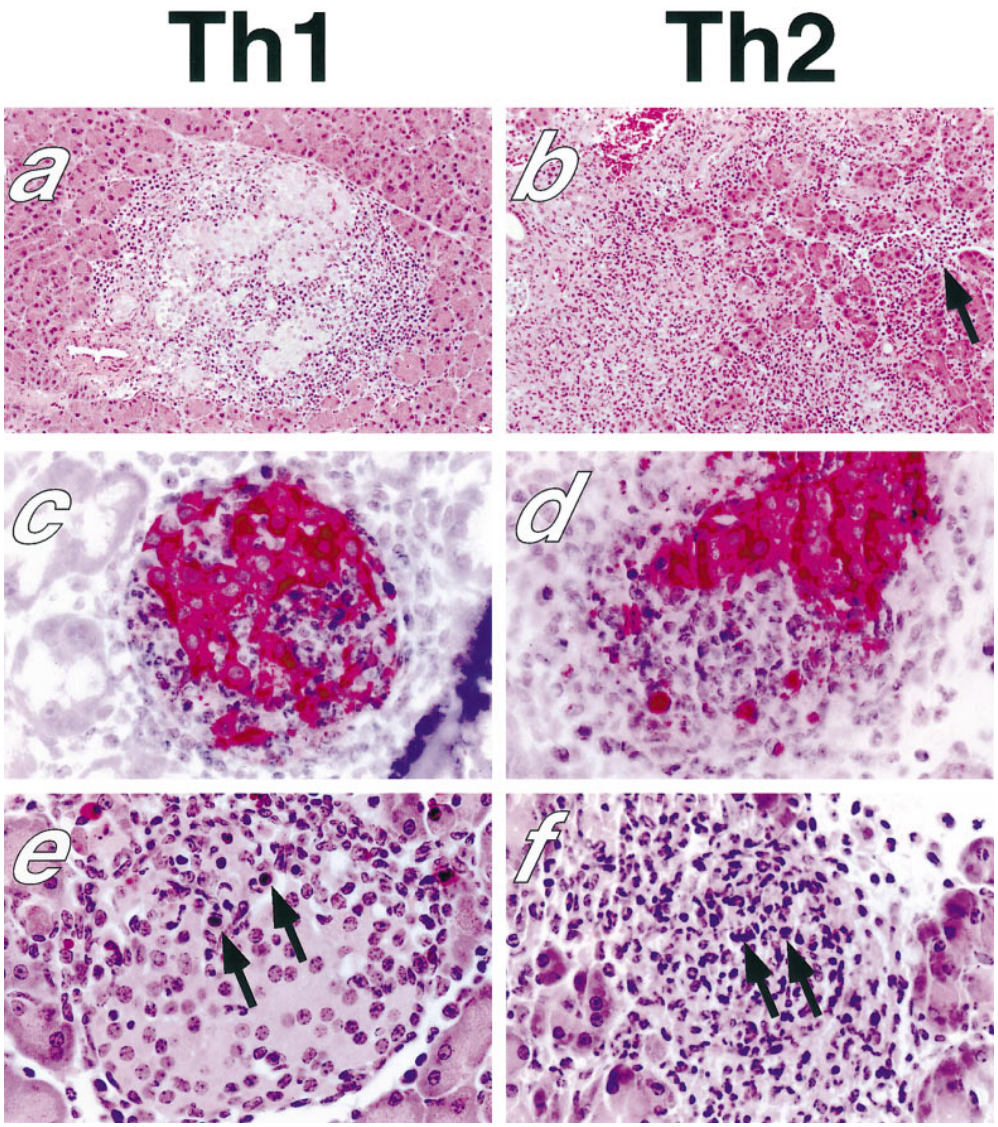

Figure 2. Distinct lesions created by Th1 and Th2 cells in NOD.*scid* mice. Photomicrographs of pancreatic tissue sections stained with hematoxylin and eosin, (*a*) Th1, and (*b*) Th2. Th1 lesion shows confined periinsulitis and insulitis pattern with little exocrine inflammation and damage, whereas Th2 lesion shows swarming pancreatitis with infiltration of the exocrine and endocrine tissue. Arrow in *b* indicates exocrine infiltration and damage. Pancreatic sections stained with anti-insulin (*AEC*) counterstained with hematoxylin reveals β cell function; Th1 (*c*) and Th2 (*d*). Th1 lesion shows the outside–in pattern of islet destruction with intact insulin-producing β cells in the center of the islet, whereas Th2 lesion shows characteristic asymmetric destruction of islet by necrosis. Th1 lesion stained for TUNEL$^+$ nuclei (*DAB*) and counterstained with hematoxylin and eosin (*e*). Arrows indicate apoptotic β cells. Hematoxylin and eosin staining of a Th2 lesion shows a necrotic islet mass containing eosinophils (*arrows*) and other PMN and cell debris (*f*). Original magnifications: *a* and *b*, 50×; *c–f*, 100×.

also the exocrine tissue (Fig. 2 *b*) with significant damage produced to the exocrine tissue as well as islets (Fig. 2, *b* and *f*). The Th2 lesions contained a swarming pancreatitis predominantly composed of eosinophils and PMN cells (Fig. 2 *f*). Additionally, the Th2 lesions were characterized by the presence of abscesses as evidenced by the massive accumulation of eosinophils and PMNs with necrotic cellular debris with in the islets (Fig. 2, *b* and *f*).

The organized outside–in pattern of Th1 T cell–mediated infiltration was associated with β cell apoptosis. Insulin-containing β cells were present in the intact areas of infiltrated islets (Fig. 2 *c*), whereas infiltrated areas saw a loss of insulin-bearing islet cells. Th1 lesions showed β cells apoptosis (Fig. 2 *e*) similar to that seen in spontaneous disease (8). There was a strict correlation between the presence of insulitis and and β cell apoptosis. Cell death in the Th2 cell infiltrated islets by contrast was due largely to necrosis and subsequent abscess formation (Fig. 2 *e*). It is worth noting that the islet cell necrosis in Th2 lesions was often asymmetric or segmental especially with large islets where one portion of the islet would be completely necrotic and the adjacent islet mass completely intact and producing insulin (Fig. 2 *d*).

Dramatic differences in the organization and cellular makeup of the two lesions was further underscored upon immunohistochemical labeling of pancreatic frozen sections from NOD.*scid* mice infused with Th1 and Th2 T cells. Fig. 3 *a* depicts a section of a Th1 T cell–induced lesion, showing an accumulation of transgenic Vβ4$^+$ T cells peripheral to the infiltrated islet. The exocrine tissue was largely devoid of any T cells. By contrast, in Th2 lesions, abscess formation was seen with a few weakly staining CD4$^+$, Vβ4$^+$ T cells associated with both islet and the peripheral exocrine tissue (Fig. 3 *b*). In general, T cell staining was weak and diffuse and made up only a small portion of the total leukocytic infiltration within the inflamed pan-

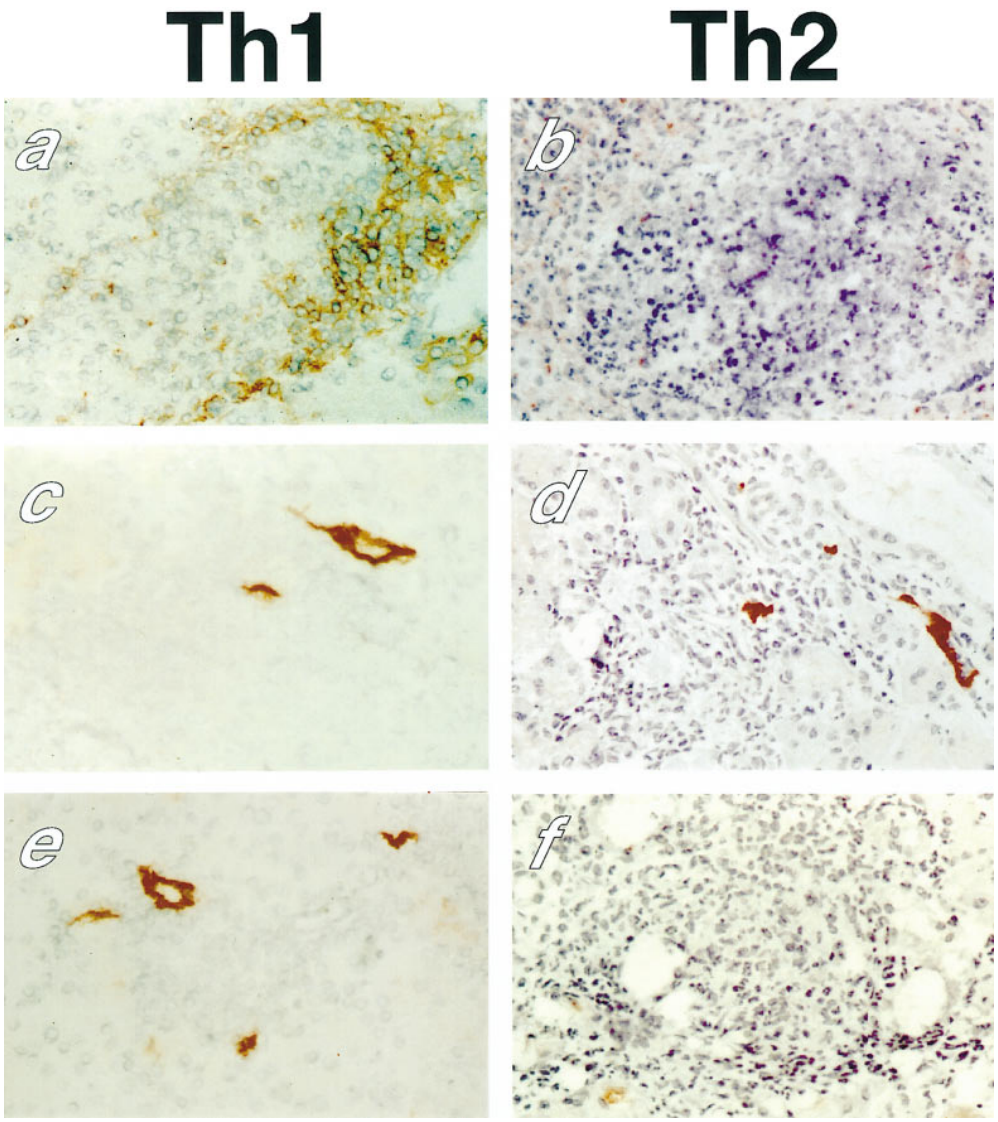

Figure 3. Immunohistochemistry on pancreatic sections from NOD.*scid* mice transferred with Th1 or Th2 cell. Th1 T cell lesion stained for anti-Vβ4 shows organized transgenic T cell infiltration surrounding islet (*a*). Th2 lesion stained for anti-Vβ4 shows scattered T cell localization and central necrotic mass with cell debris (*b*). *MECA-367* mAb staining to reveal mad-CAM expression on high endothelial venule in both Th1 (*c*) and Th2 (*d*), lesions. MECA-79 mAb staining to reveal peripheral node addressin on HEV. PNA staining is present on HEV from Th1 lesion (*e*), but lacking on HEV from Th2 lesion (*f*). All photomicrograph original magnifications: 40×. Staining revealed by AEC and counterstained in hematoxylin.

creas. Macrophage subpopulations were present in both Th1 and Th2 lesion as ascertained by CD11b, MOMA-1, MOMA-2, and ER-MP23 staining (data not shown). However, the Th2 lesions had a larger number of scavenger macrophages surrounding the islet abscesses.

Peripheral Node Addressin Is Not Expressed in the High Endothelial Venules of Th2 Recipient Mice. Both Th1 and Th2 lesions showed mad-CAM on the high endothelial venules with in the pancreas (Fig. 3, *c–d*). However, Th2 lesions lacked expression of the activated form, peripheral node addressin, PNA, as detected by the MECA-79 mAb (Fig. 3, *e–f*). This was significant because PNA expression requires localized production of IFN-γ and TNF-α, again suggesting that Th2 lesions lack significant production of these Th1 cytokines.

IL-10 Antibody Treatment Protects NOD.scid Mice from Th2-mediated Diabetes. To rule out the contribution of neutrophils and granulocytes in the pancreatitis associated with Th2 T cell transfer, we treated mice with a granulocyte-specific mAb RB6 at concentrations sufficient to severely deplete granulocytes in vivo (24, 25). We found no change in disease or in the characteristics of the Th2 lesion when granulocytes and neutrophils were depleted from mice injected with Th2 cells (data not shown). However, when we treated Th2 recipient NOD.*scid* mice with neutralizing antibodies against IL-10, a Th2 cytokine previously shown to have a major effect on autoimmune diabetes, the Th2-mediated disease was significantly ameliorated. This was not true for another Th2 cytokine, IL-4 (Fig. 4). NOD.*scid* mice received either 250 μg of anti–IL-4, anti–IL-10, or saline every 72 h, with the first dose 24 h before infusion of the Th2 T cells. As depicted in Fig. 4, anti–IL-4 treatment did not protect from diabetes, whereas anti–IL-10 significantly delayed or abrogated the Th2 pathology. Histology performed on pancreas from these mice indicate no change in the lesion characteristics, yet anti–IL-10 treat-

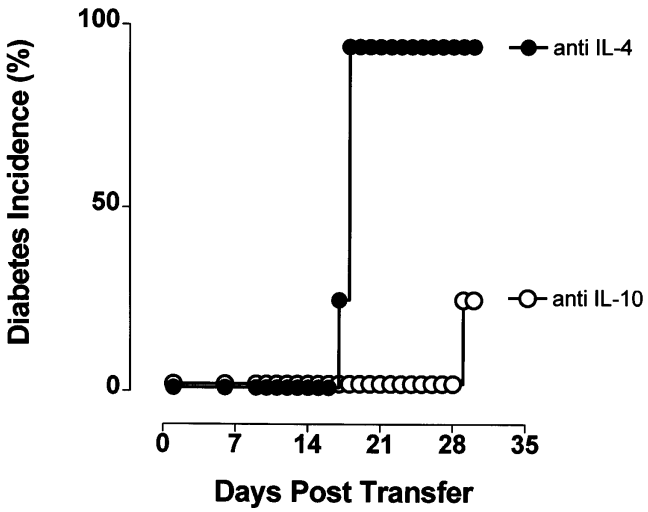

Figure 4. Anti–IL-10 mAb treatment protects NOD.*scid* mice from Th2-mediated disease. Mice were treated with 250 μg of purified mAb against IL-10 (2A5) and IL-4 (11B11) every 72 h. Open circles represent anti–IL-10–treated mice and closed circles represent anti–IL-4–treated mice. Diabetes was assessed by measuring blood glucose. Mice with two consecutive blood sugar readings above 250 mg/dl were considered diabetic. Onset was dated to the first of the two consecutive readings.

ment substantially protected against Th2 lesions, suggesting that IL-10 contributes to the pathology of Th2 disease.

Discussion

We reported that Th2 cells were capable of inducing hyperglycemia in immune-compromised NOD.*scid* mice but not in neonatal NOD mice. The kinetics of disease was slower when compared with Th1-mediated disease due to a different mechanism of islet cell destruction. The Th2 lesions were characterized by a predominantly eosinophilic infiltration, islet necrosis and abscess, and a severe pancreatitis with destruction of both exo- and endocrine tissue. By contrast, Th1 T cells produced focally confined infiltration of the islets and β cell apoptosis which largely spares the adjacent exocrine tissue. This was more reminiscent of the lesions seen in the natural disease and in our spontaneous TCR transgenic model of disease (8, 12).

Two important question arise from these findings. First, how do Th2 T cells propagate the pancreatitis and necrosis of islet cells in the immune-compromised recipient? And, second, why is the Th2 lesion found exclusively in immune-compromised hosts? Addressing the former, our data suggest that a particularly important mediator of islet cell necrosis is IL-10. Interestingly, previously published reports using transgenic mice that produced IL-4 in the islets were protected from diabetes, whereas local production of IL-10 produced a severe disease (26–28). These observations agree well with our findings that anti–IL-10 treatment of Th2 T cell recipients greatly diminishes disease onset and concurrent pathology. Wogensen et al. (26) have suggested that localized production of IL-10 produces important changes in the vascular endothelium, which may lead to accumulation of T cells, macrophage and eosinophils by changing the vascular addressin expressed on the endothelium. However, if the local production of IL-10 by our Th2 T cells acted predominantly to stimulate homing, treatment of the recipients with anti–IL-10 should have diminished greatly the eosinophilic nature of infiltration. However, we found that anti–IL-10 had a far greater effect on the pathology of the lesion than on its cellularity. One hypothesis is that IL-10 leads to vascular damage resulting in hypoxia and subsequent abscess formation. Moritani et al. (28) have previously described ductal proliferation as an effect mediated by transgenic expression of IL-10. Alternatively, owing to the venation of microcapillaries that feed islets (29), localized endothelial damage produced by IL-10 could lead to vascular occlusion of one of the branches feeding the microvascular circulation of the islet, resulting in the segmented necrosis that affected many of the larger islets. However, we have not found any direct evidence to support this interpretation.

The susceptibility of immune-compromised animals to Th2-mediated disease may lie with the activated state of the innate immune system in the absence of specific immunity. Specifically, IL-10 secretion has pronounced effects on mononuclear phagocyte development and can enhance the development of the myelomonocytic lineage (30–32). However, the most likely explanation rests with the lack of additional αβ–T cells. It is likely that Th2 cell regulation requires interactions with other αβ–T cells. Because in both our results and those of Lafaille et al. (33), the exaggerated and deleterious Th2 phenotype occurs only in the absence of diverse αβ–T cell compartment, how the presence of other T cells affect the in vivo function of Th2 remains to be seen.

It would be of obvious clinical advantage if one could deviate an ongoing inflammatory immune response to a benign Th2 response. Some have even suggested that Th2 T cells are suppressive or tolerant T cells, reviewed and discussed in references 11, 34. But it is clear that some doubts have existed as to the efficacious use of Th2 therapy to treat inflammatory-based diseases (35–37). Our results demonstrate that Th2 cells may not be all that benign, especially if pushed to extremes, and carry with them a real potential to cause clinical disease. This is of particular concern in the case at hand, autoimmune diabetes, where immune deviation therapy as proposed in conjunction with islet cell transplantation and immunosuppression may cause a severe necrotic destruction of engrafted islets. Moreover, this may not be a limited phenomenon as similar studies in experimental autoimmune encephalomyelitis yield remarkably similar results (33).

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Address correspondence to J.D. Katz, Department of Pathology and Center for Immunology, Washington University School of Medicine, Campus Box 8118, 660 South Euclid, St. Louis, Missouri 63110. Phone: 314-747-1221; Fax: 314-747-0728; E-mail: jkatz@immunology.wustl.edu

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