T-Cell Production of an Inducible Interleukin-10 Transgene Provides Limited Protection From Autoimmune Diabetes

Mary E. Pauza, Heather Neal, Amy Hagenbaugh, Hilde Cheroutre, and David Lo

In a number of animal models of spontaneous autoimmune diabetes, pathogenesis has been highly correlated with autoreactive T-cell production of the type 1 cytokine interferon-γ (IFN-γ), while protection from disease was associated with type 2 cytokines such as interleukin (IL)-4. Curiously, in some models, diabetes is associated with unexpected cytokine patterns; for example, diabetes can develop in NOD mice lacking a functional IFN-γ gene. In another situation, acceleration of diabetes occurs in transgenic mice with constitutive β-cell expression of the type 2 cytokine IL-10. IL-10 has generally been associated with immunosuppression, including the modulation of class II expression on antigen-presenting cells and the generation of regulatory CD4 T-cells. Because it is possible that unregulated expression of any cytokine might lead to unphysiological effects in vivo, we tested the notion that an inducible T-cell–specific IL-10 transgene might yet mediate a more physiological protection from autoimmune diabetes. Our results show that indeed, regulated T-cell production of IL-10 does not accelerate diabetes and instead can provide significant protection from disease. These results help rectify the apparent discrepancies between the effect of IL-10 on various models of autoimmune diabetes. *Diabetes* 48:1948–1953, 1999

Cytokines with immunosuppressive capacities, such as interleukin (IL)-10, have been the focus of recent investigation for their potential in treating immunologic disorders. Application of IL-10 has resulted in disease improvement in autoimmune models of rheumatoid arthritis, inflammatory bowel disease, and autoimmune encephalomyelitis (1–3). IL-10 is believed to function in these models either by enhancing production of regulatory/suppressor cells or by suppressing T-cell effector functions (1–4). Additional effects of IL-10 have been observed on antigen-presenting cells, such as modulation of class II major histocompatibility expression, that may contribute to the changes observed in lymphocyte function in these disease models (5,6). In spite of the immunosuppressive effects attributed to IL-10, its in vivo ability to modulate autoimmune diabetes remains paradoxical (7–10). In NOD mice, IL-10 overexpression in the pancreas islet α- or β-cells accelerates the onset of diabetes (11,12). On the other hand, numerous correlations have been made between autoimmune diabetes attenuation and increased IL-10 production, suggesting that IL-10 has a protective effect (13–16). In addition, systemic administration of IL-10 by various means (e.g., direct cytokine, immunoglobulin fusion protein, or injected expression plasmid administration) can result in protection from disease (17–19).

The seemingly contradictory effects of IL-10 on autoimmune diabetes may be the result of differences inherent in the models under study. These include differences in local versus systemic cytokine administration/production, constitutive versus regulated expression, differences in cytokine concentration achieved in vivo, differences in developmental timing of administration/production of cytokine, or a combination of these. To better understand this dichotomy, we have studied the effect of regulated IL-10 production by T-cells on diabetes incidence in both spontaneous and adoptive transfer models of disease. In our models, transgene-mediated IL-10 production is controlled by the human interleukin-2 (hIL-2) promoter, resulting in expression by T-cells only after activation. Our data suggest that regulated expression of IL-10 by T-cells does not result in autoimmune diabetes acceleration; rather, under these more controlled conditions, IL-10 actually delays disease.

From the Department of Immunology (M.E.P., H.N., D.L.), The Scripps Research Institute, La Jolla; and the La Jolla Institute for Allergy and Immunology (A.H., H.C.), San Diego, California.

Address correspondence and reprint requests to David Lo, Department of Immunology IMM-25, The Scripps Research Institute, 10550 North Torrey Pines Rd., La Jolla, CA 92037. E-mail: davidlo@scripps.edu.

Received for publication 20 April 1999 and accepted in revised form 6 June 1999.

ELISA, enzyme-linked immunosorbent assay; HA, hemagglutinin; HBSS, Hanks' balanced salt solution; hIL-2, human interleukin-2; HPRT, hypoxanthine-guanine phosphoribosyl transferase; IFN, interferon; IL, interleukin; RIP-IL-10-NOD mice, NOD mice expressing a rat insulin promoter–driven IL-10 transgene; RT-PCR, reverse transcriptase–polymerase chain reaction; TCR, T-cell receptor; Th, T-helper; TSRI, The Scripps Research Institute.

RESEARCH DESIGN AND METHODS

Mice. Mice bearing a murine IL-10 genomic transgene controlled by the human IL-2 promoter-enhancer region have been previously described (20). IL-10 transgenic mice were crossed with B10.D2 mice (The Scripps Research Institute [TSRI] rodent breeding colony, La Jolla, CA) until homozygosity was achieved at the major histocompatibility locus (H-2^d) as determined by polymerase chain reaction of tail DNA using microsatellite marker MM23 primers (sense: 5′-GTTTCAGT TCTCAGGGTCCTA-3′, anti-sense: 5′-CAGGATTCTGTGGCAATCTGG-3′). Mice expressing a T-cell receptor (TCR)-SFE specific for the hemagglutinin (HA) peptide 110–119 (SFERFEIFPK) presented by I-E^d have been previously characterized (21). For adoptive transfer experiments, IL-10 transgenic mice crossed with TCR-SFE transgenic mice provided a source of IL-10/TCR-SFE transgenic donor T-cells. In addition, IL-10/TCR-SFE mice were crossed to Ins-HA transgenic mice as a model of spontaneous type 1 diabetes (IL-10/TCR-SFE/Ins-HA). In Ins-HA trans-

Downloaded from http://diabetesjournals.org/diabetes/article-pdf/48/10/1948/363756/10512358.pdf by guest on 11 October 2024

genic mice, HA is expressed by islet β-cells under the control of the rat insulin promoter (22). Separately, IL-10 transgenic mice were backcrossed with NOD/Shi mice (TSRI rodent breeding colony) for seven generations (NOD.IL-10). All mice were maintained in a virus-free environment at TSRI rodent colony in accordance with National Institutes of Health and TSRI institutional guidelines.

Reverse transcriptase–polymerase chain reaction. Transgene expression of IL-10 was specifically assayed by reverse transcriptase–polymerase chain reaction (RT-PCR). Lymph node mononuclear cells were isolated from IL-10 transgenic mice by density gradient centrifugation with Lympholyte M (Accurate Hornby, Ontario, Canada). Cells were stimulated on anti-CD3ε (10 μg/ml) plus anti-CD28 (1 μg/ml) coated plates overnight at 37°C, 5% CO_2. Total RNA was isolated from cultured cells using Trizol (Life Technologies, Rockville, MD) according to the manufacturer's protocol. First-strand cDNA was synthesized from RNA using oligo-dT primers with the Superscript II kit (Life Technologies) according to the manufacturer. PCR of cDNA was performed using the following primer pairs: hIL-2 promoter (IL-10 sense) 5′-CCCTATCACTCTTTAATCACTACTCA-3′, IL-10 anti-sense 5′-ATTTCCGATAAGGCTTGGCAACCC-3′ (380 bp); interferon (IFN)-γ sense 5′-CATTGAAAGCCTAGAAAGTCTG-3′, IFN-γ anti-sense 5′-CTCATGAATGCAT CCTTTTTCG-3′ (275 bp); hypoxanthine-guanine phosphoribosyl transferase (HPRT) sense 5′-GTTGGATACAGGCCAGACTTTGTT-3′, and HPRT anti-sense 5′-GAGGGTAGGCTGGCCTATAGGCT-3′ (350 bp). Analysis of IL-10 expression was specific for transgene-derived cytokine because the sense primer is derived from the 5′ untranslated region of the human IL-2 promoter region, and the anti-sense primer is from the third exon of murine IL-10. Lymph node cells from transgene-negative mice were used as controls for IL-10 transgene expression.

Adoptive transfers. Spleen and lymph node mononuclear cells were pooled from IL-10/TCR-SFE or TCR-SFE mice. The total number of $CD4^+$ T-cells in each pool was determined by multiplying the total cell yield with the percentage of $CD4^+$ cells as determined by flow cytometry using anti-CD4-phycoerythrin (PharMingen, La Jolla, CA). Adoptive transfers of 1×10^7 $CD4^+$ T-cells were performed by intravenous injections into irradiated (700 rad) Ins-HA recipients. The total cell numbers varied for each donor group, but CD4 T-cell numbers were kept constant for each recipient injection. Blood glucose levels were monitored weekly after cell transfer.

Diabetes monitoring and statistics. Onset of diabetes (16.6 mmol/l) was determined by monitoring blood glucose levels using Chemstrip bG test strips with an Accu-Chek III blood glucose monitor (Boehringer Mannheim, Indianapolis, IN). Incidence of diabetes was graphed on Kaplan-Meier cumulative survival plots and the log-rank (Mantel-Cox) test for statistical significance was performed using StatView software.

Proliferation assays and enzyme-linked immunosorbent assay. Spleen and lymph node mononuclear cells were isolated from Ins-HA, IL-10/Ins-HA, TCR-SFE/Ins-HA, and IL-10/TCR-SFE/Ins-HA mice. For proliferation assays, 5×10^5 lymph node or 1×10^6 spleen cells/well were stimulated with plate bound anti-CD3ε (10 μg/ml) plus anti-CD28 (1 μg/ml) for 48 h and pulsed with 1 μCi/well for another 24 h before harvest. Triplicate samples were counted on a Microbeta Trilux liquid scintillation counter (Wallac, Turku, Finland). In parallel, lymph node and spleen cells were stimulated as described above in a total volume of 200 μl/well. Supernatants were collected after 48 h and assayed for IFN-γ by sandwich enzyme-linked immunosorbent assay (ELISA) using anti–IFN-γ (PharMingen) and peroxidase-conjugated streptavidin plus 2,2′-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid). All incubations were for 30 min at 37°C. Plates were washed with phosphate-buffered saline containing 0.05% Tween 20 after each incubation. IL-10 was assayed using an IL-10 ELISA kit (BioSource, Camarillo, CA) following the manufacturer's instructions. Cytokine concentrations were interpolated from standard curves obtained from spectrophotometer (Spectra 2.06; BioMetallics, Princeton, NJ) readings using DeltaSoft 3 software.

Flow cytometry. Islet tissue was obtained from pancreases of B10.D2, TCR-SFE/Ins-HA, and IL-10/TCR-SFE/Ins-HA mice by partial collagenase digestion (23). Briefly, pancreas tissue was infused with 1 mg/ml collagenase P (Boehringer Mannheim) in Hanks' balanced salt solution (HBSS) without calcium or magnesium, cut into approximately six pieces, and digested at 37°C, 125 rpm for 20 min. Islets were handpicked using a dissecting microscope, washed in HBSS, and dissociated with frosted glass slides before staining with anti-B220–fluorescein isothiocyanate, anti-CD4–phycoerythrin, and anti-CD8–allophycocyanin (PharMingen). Stained cells were analyzed on a FACS Calibur (Becton Dickinson, Franklin Lakes, NJ) with Cell Quest version 3.2 software (Becton Dickinson).

RESULTS

The previously reported effects of IL-10 on autoimmune diabetes fall into two categories. Where IL-10 accelerated diabetes, disease depended on IL-10 constitutive expression within the islets of Langerhans (11,24–26). Conversely, where IL-10 attenuated disease, systemic forms of IL-10 were given.

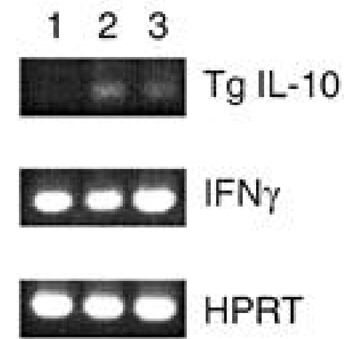

FIG. 1. Transgenic IL-10 is expressed within 24 h after stimulation of lymphocytes from IL-10 transgenic mice. RT-PCR was performed on RNA from lymph node mononuclear cells of nontransgenic (*lane 1*) or IL-10 transgenic (*lanes 2* and *3*) mice after overnight stimulation with anti-CD3 plus anti-CD28. Primers used for each RT-PCR are indicated (*right*). Primers used to detect IL-10 are specific for transgenic IL-10 (not endogenous). Amplification of IFN-γ indicates that lymphocyte populations were effectively stimulated for all samples, and HPRT message shows equal cDNA concentrations were used in each reaction.

Both types of studies are far removed from the physiologic expression of IL-10, which is typically produced by activated T-cells, macrophages, or B-cells (8,9). Therefore, we have chosen to examine the effects of overexpression of IL-10 in a more regulated and physiologic transgenic model where murine IL-10 expression is directed in T-cells by the human IL-2 promoter. Transgenic IL-10 expression mimics IL-2 in that it is rapidly upregulated after TCR-mediated stimulation and is detectable by RT-PCR within 24 h (Fig. 1). This expression differs from endogenous IL-10 production by naive T-cells, which occurs late after T-cell stimulation; however, transgene IL-10 production is more characteristic of differentiated T-helper (Th) 2-cells (20,27).

Regulated IL-10 expression does not accelerate diabetes onset in NOD mice. To determine if expression of transgenic IL-10 by T-cells could influence the onset of spontaneous autoimmune diabetes, IL-10 transgenic mice were backcrossed with NOD mice for seven generations. Onset of diabetes (16.6 mmol/l) was followed by monitoring blood glucose levels of female mice every 2 weeks. No significant inhibition in disease onset was observed in NOD.IL-10 compared with nontransgenic NOD control mice (Fig. 2*A*). Importantly, no acceleration in disease onset was observed unlike previous studies in which IL-10 was expressed directly by islet cells (11,24–26).

Diabetes onset is significantly delayed in a transgenic model of diabetes. In NOD mice, the specificity of autoreactive T-cells is not well defined. To examine the effects of the IL-10 transgene in a more defined system, we used a transgenic model of diabetes in which a defined T-cell receptor transgene (TCR-SFE) is combined with β-cell–specific expression of the target antigen influenza hemagglutinin (Ins-HA). We first examined the effect of IL-10 on adoptively transferred disease using IL-10/TCR-SFE donor T-cells and Ins-HA recipients. While the onset of hyperglycemia in the spontaneous disease of NOD mice typically occurs at 3–8 months of age, it is more rapid in adoptive transfer models,

Downloaded from http://diabetesjournals.org/diabetes/article-pdf/48/10/1948/363756/10512358.pdf by guest on 11 October 2024

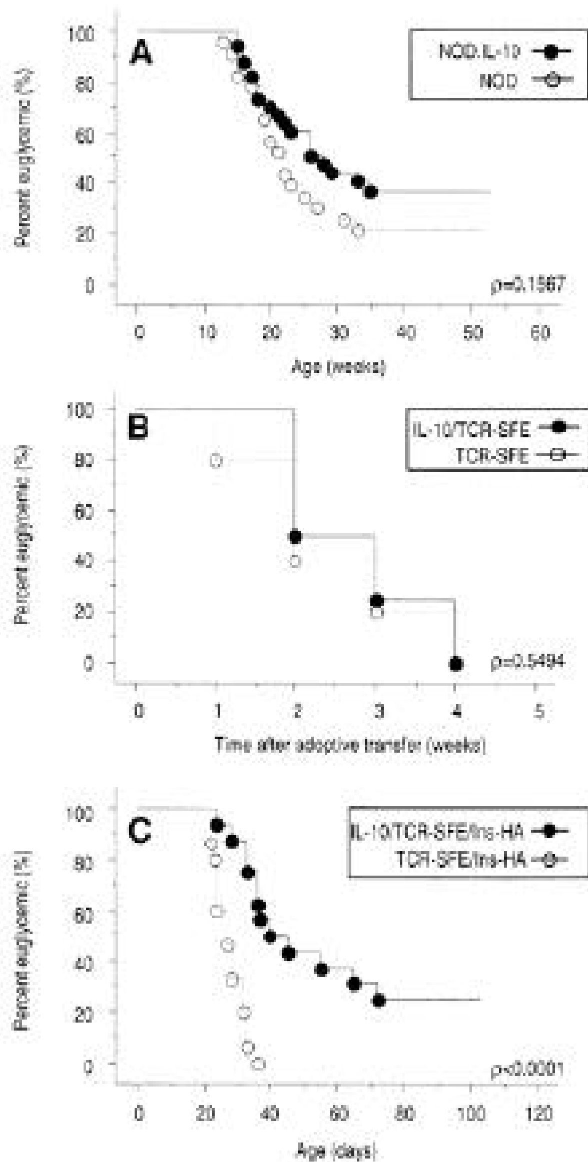

FIG. 2. Transgenic IL-10 expression significantly inhibits the onset of spontaneous diabetes. Onset of diabetes was followed in three models: NOD (*A*), adoptive transfer (*B*), and spontaneous transgenic (*C*) models. Diabetes was determined by measuring blood glucose levels every 7 days (adoptive transfer and spontaneous transgenic models) or 2 weeks (NOD). Kaplan-Meier plots compare the onset of diabetes between IL-10 transgenic and control mice over the course of study for each model. Data represent 35 (NOD.IL-10), 24 (NOD), 5 (IL-10/TCR-SFE), 4 (TCR-SFE), 16 (IL-10/TCR-SFE/Ins-HA), and 15 (TCR-SFE/Ins-HA) mice. Only female mice were included in the analysis of NOD models. One representative experiment from a total of five adoptive transfer experiments is shown. *P* values are indicated for each model reflecting log-rank (Mantel-Cox) analysis.

occurring within 4 weeks of transfer (21,28). When IL-10/TCR-SFE T-cells were adoptively transferred into irradiated Ins-HA recipients, an aggressive disease indistinguishable from control TCR-SFE cell transfers was observed (Fig. 2*B*). Because of the rapid kinetics of adoptively transferred disease, any accelerating effects of IL-10 overexpression may be difficult to detect in this model, but regulated overexpression of IL-10 also did not suppress disease onset.

Spontaneous disease will also develop in double transgenic TCR-SFE/Ins-HA mice (21,28). These mice become diabetic rapidly after birth by virtue of antigen-specific CD4 T-cell–mediated β-cell destruction, with up to 100% of mice becoming diabetic within 40 days (Fig. 2*C*). However, despite the rapid kinetics observed, the islet inflammation closely resembles the well-organized infiltrates of spontaneous disease of NOD mice (21). In contrast to the adoptive transfer model, the kinetics of islet inflammation may be more gradual as the immune system develops in the perinatal period. Interestingly, in this case, addition of the IL-10 transgene (IL-10/TCR-SFE/Ins-HA) to this model caused a significant delay in onset of hyperglycemia.

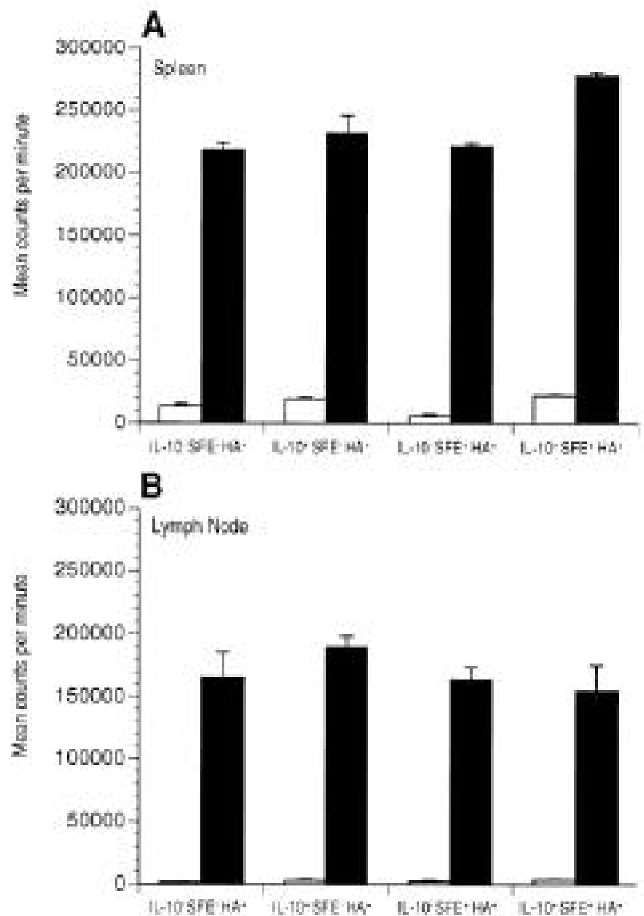

FIG. 3. IL-10 transgenic T-cells proliferate normally to anti-CD3 plus anti-CD28 stimulation. Spleen (*A*) or lymph node (*B*) mononuclear cells isolated from Ins-HA, IL-10/Ins-HA, TCR-SFE/Ins-HA, and IL-10/TCR-SFE/Ins-HA mice were stimulated with anti-CD3 plus anti-CD28 (■) or media alone (□). Mean values of triplicate samples are shown with error bars representing 1 SD from the mean. Results are representative of individual mice from one of two separate experiments with *n* = 2–5 mice/genotype.

Delayed diabetes onset in IL-10/TCR-SFE/Ins-HA triple transgenic mice could reflect a direct effect of the IL-10 transgene on T-cell functions such as proliferation or Th1/Th2 commitment. However, in vitro proliferative responses to anti-CD3 plus anti-CD28 were not different among triple transgenic spleen or lymph node cells compared with single (Ins-HA) or double (TCR-SFE/Ins-HA) transgenic cells (Fig. 3). Likewise, IFN-γ responses of triple transgenic T-cells to anti-CD3 plus anti-CD28 remained similar to those of controls (Fig. 4*A*). These data agree with reports that IL-10 alone does not affect T-cell proliferation or cytokine production directly

Downloaded from http://diabetesjournals.org/diabetes/article-pdf/48/10/1948/363756/10512358.pdf by guest on 11 October 2024

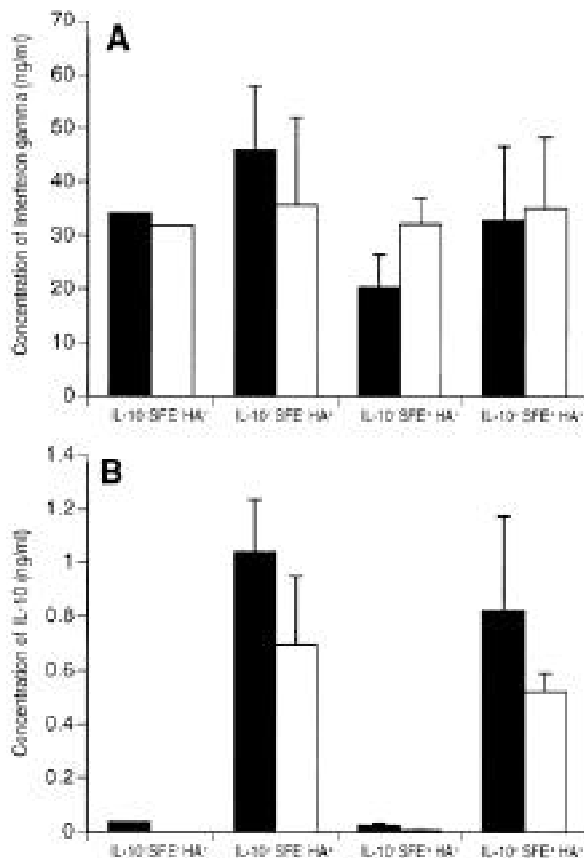

FIG. 4. IL-10 transgene expression does not inhibit Th1-cell development as measured by IFN-γ production in response to anti-CD3 plus anti-CD28 stimulation. Spleen (■) or lymph node (□) cells were isolated and stimulated as described in Fig. 3. Culture supernatants (48 h) were analyzed for the presence of IFN-γ (*A*) and IL-10 (*B*) by ELISA. Results are mean cytokine concentrations observed in two separate experiments with $n = 3–4$ mice/genotype, except for Ins-HA mice, where only mean values are shown because $n = 2$ mice.

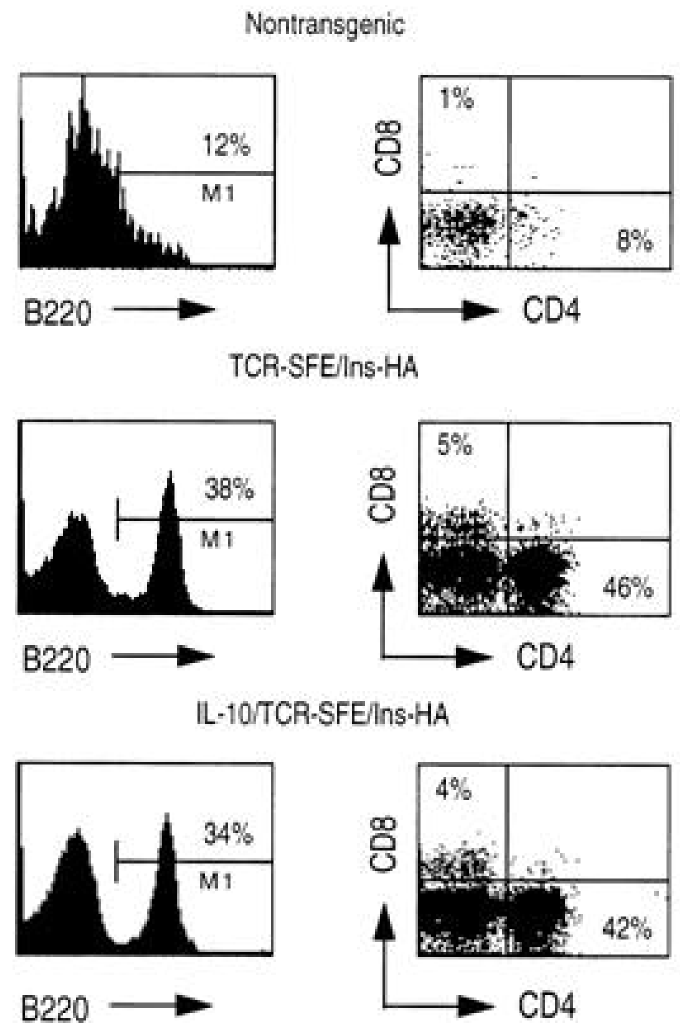

FIG. 5. IL-10 expression does not change lymphocyte recruitment into islet tissue in triple transgenic mice. Single-cell suspensions were isolated from islet tissue of nontransgenic, TCR-SFE/Ins-HA, and IL-10/TCR-SFE/Ins-HA mice. Cells were analyzed by flow cytometry for the presence of B-cells (B220) and T-cells (CD4 and CD8). Only events within the typical lymphocyte forward and side scatter gate (as determined with lymph node cells) were included in this analysis. Because nontransgenic mice have normal islet tissue, very few lymphocytes could be detected in pancreases from these mice. Marker and quadrant settings were based on staining with isotype-matched control antibodies. Percentages of $B220^+$, $CD4^+$, and $CD8^+$ cells are indicated in each plot. Inguinal lymph node mononuclear cells were analyzed in parallel: nontransgenic, 26% $B220^+$, 27% $CD8^+$, 46% $CD4^+$; TCR-SFE/Ins-HA, 38% $B220^+$, 9% $CD8^+$, 51% $CD4^+$; IL-10/TCR-SFE/Ins-HA, 47% $B220^+$, 7% $CD8^+$, 43% $CD4^+$.

(5,8,9,29,30). Instead, IL-10 has been shown to enhance T-cell proliferation and CD8 cytotoxic T lymphocyte (CTL) precursor frequency and activity only when present in addition to other cytokines such as IL-2 and IL-4 (16,31). IL-10 is also known to have indirect effects on T-cell proliferation and cytokine production via changes in antigen-presenting cell functions such as class II expression (6). In a previous study, this IL-10 transgene was unable to convert the protective Th1 response of C57BL/6 mice to Leishmania major infection to a Th2 response (20). Thus, the delay in diabetes onset observed among IL-10 transgenic mice was likely independent of any direct inhibition of T-cell proliferation by IL-10 and was not due to Th2 commitment of all CD4 T-cells (Fig. 4*A* and *B*).

In previous reports on mice with constitutive islet β-cell expression of IL-10, striking patterns of islet inflammation were observed, mainly dominated by B-cells (24–26). To determine whether related alterations in islet inflammation could be induced by T-cell expression of an IL-10 transgene, we examined lymphocyte recruitment patterns in the islets of TCR-SFE/Ins-HA and IL-10/TCR-SFE/Ins-HA mice. Because the SFE-TCR is class II restricted, peripheral T-cells in TCR-SFE transgenic mice are mainly $CD4^+$ (21). Consequently, the lymphocytes surrounding islet tissue in TCR-SFE/Ins-HA mice are primarily B-cells and $CD4^+$ T-cells. Importantly, while transgenic T-cells produced high levels of IL-10 (Fig. 4*B*), in vivo production of IL-10 did not result in profound changes in islet lymphocyte accumulation (Fig. 5). Thus, the lymphocyte composition in IL-10/TCR-SFE/Ins-HA mice was essentially identical to that seen in double transgenic mice indicating that IL-10 did not influence gross lymphocyte subset recruitment to the islets. Together, these data suggest that the delay in diabetes onset mediated by T-cell overexpression of IL-10 was due to more subtle effects on disease pathogenesis.

DISCUSSION

Despite the paradoxical effects attributed to IL-10 by various in vivo studies, it is apparent from the present study that the

Downloaded from http://diabetesjournals.org/diabetes/article-pdf/48/10/1948/363756/10512358.pdf by guest on 11 October 2024

location and regulation of IL-10 expression can bias its influence on disease outcome. The delay in diabetes onset observed in IL-10/TCR-SFE/Ins-HA transgenic mice agrees with previous studies where systemically administered IL-10 resulted in suppression of diabetes (17–19). However, our results are in stark contrast to the acceleration of diabetes observed with constitutive islet cell overexpression of IL-10 (NOD mice expressing a rat insulin promoter–driven IL-10 transgene [RIP-IL-10-NOD]) (25). Together, these data suggest that IL-10 will suppress diabetes onset, but this is dependent on appropriately regulated expression.

Why does IL-10 result in disease acceleration in RIP-IL-10-NOD mice but not NOD.IL-10 mice? Constitutive local IL-10 expression may contribute to disease acceleration in RIP-IL-10-NOD mice in an autoreactive lymphocyte-independent manner. These effects are most obvious in diabetes-resistant BALB/c mice, where constitutive expression of IL-10 results in activation of islet vascular endothelium with increased levels of peripheral node addressin, intercellular adhesion molecule (ICAM)-1, and von Willebrand factor (12) and is associated with significant peri-insulitis. By contrast, in our single transgenic IL-10 mice, the IL-2 promoter-regulated T-cell–specific expression of IL-10 did not cause peri-insulitis (20 and data not shown). Moreover, this transgene was able to inhibit inflammation in other disease models such as inflammatory bowel disease (20).

When attempting to understand the role that regulated IL-10 overexpression might have in both NOD and transgenic spontaneous diabetes models, it becomes important to consider the differences between these models. Diabetes development in NOD mice requires both CD4 and CD8 cells, while our transgenic model is independent of CD8 cells. Because no significant effect was observed in the NOD model, it is possible that regulated IL-10 overexpression does not affect CD4 and CD8 cells equally. Studies by Jinquan et al. (32) indicate that IL-10 is selectively chemotactic for CD8 cells. Chen and Zlotnik (31) have also shown that IL-10 enhances cytotoxic T-cell differentiation and effector functions. In addition, the accelerating effect of constitutive IL-10 overexpression in RIP-IL-10-NOD mice was clearly dependent on CD8 cells (33). In contrast, IL-10 inhibits antigen-presenting cell–mediated cytokine production and Th1 commitment by CD4 cells (5,29,30,34). Together, these data suggest that IL-10 might serve to stimulate CD8 cell trafficking and function while at the same time inhibit CD4 Th1 development and function. Because diabetes in our TCR-SFE/Ins-HA transgenic model is independent of CD8 cells, any effect that IL-10 has on CD4 function will be readily observed. By contrast, in NOD mice, the inhibitory influence on CD4 cells may be counterbalanced by stimulation of CD8 cell activity. Thus, IL-10 may significantly modulate autoimmune diabetes, but its effect is dependent on intertwined factors such as location, timing, and concentration.

ACKNOWLEDGMENTS

This work was supported by the Juvenile Diabetes Foundation International, including a program project grant (D.L.) and a postdoctoral fellowship (398201) (M.E.P.), and National Institutes of Health Grants AI29689 and AI31583 (D.L.). This is publication #12388-IMM from TSRI.

The authors would like to thank Dr. Nora Sarvetnick for helpful discussions and Dr. C. Winstead for critical reading of this manuscript.

REFERENCES

1. Tanaka Y, Otsuka T, Hotokebuchi T, Miyahara H, Nakashima H, Kuga S, Nemoto Y, Niiro H, Niho Y: Effect of IL-10 on collagen-induced arthritis in mice. *Inflamm Res* 45:283–288, 1996
2. Powrie F, Leach MW, Mauza S, Menon S, Caddle LB, Coffman RL: Inhibition of Th1 responses prevents inflammatory bowel disease in scid mice reconstituted with CD45RBhi CD4+ T cells. *Immunity* 1:553–562, 1994
3. Cua DJ, Groux H, Hinton DR, Stohlman SA, Coffman RL: Transgenic interleukin 10 prevents induction of experimental autoimmune encephalomyelitis. *J Exp Med* 189:1005–1010, 1999
4. Groux H, O'Garra A, Bigler M, Rouleau M, Antonenko S, de Vries JE, Roncarolo MG: A CD4+ T-cell subset inhibits antigen-specific T-cell responses and prevents colitis. *Nature* 389:737–742, 1997
5. de Waal Malefyt R, Abrams J, Bennett B, Figdor CG, de Vries JE: Interleukin 10 (IL-10) inhibits cytokine synthesis by human monocytes: an autoregulatory role of IL-10 produced by monocytes. *J Exp Med* 174:1209–1220, 1991
6. de Waal Malefyt R, Haanen J, Spits H, Roncarolo M-G, te Velde A, Figdor C, Johnson K, Kastelein R, Yssel H, de Vries JE: Interleukin 10 (IL-10) and viral IL-10 strongly reduce antigen-specific human T cell proliferation by diminishing the antigen-presenting capacity of monocytes via downregulation of class II major histocompatibility complex expression. *J Exp Med* 174:915–924, 1991
7. MacNeil IA, Suda T, Moore KW, Mosmann TR, Zlotnik A: IL-10, a novel growth cofactor for mature and immature T cells. *J Immunol* 145:4167–4173, 1990
8. Howard M, O'Garra A, Ishida H, de Waal Malefyt R, de Vries J: Biological properties of interleukin 10. *J Clin Immunol* 14:239–247, 1992
9. de Waal Malefyt R, Yssel H, Roncarolo M-G, Spits H, de Vries JE: Interleukin-10. *Curr Opin Immunol* 4:314–320, 1992
10. Balasa B, Sarvetnick N: The paradoxical effects of interleukin 10 in the immunoregulation of autoimmune diabetes. *J Autoimmun* 9:283–286, 1996
11. Mortani M, Yoshimoto K, Tashiro F, Hashimoto C, Miyazaki J-I, Ii S, Kudo E, Iwahana H, Hayashi Y, Sano T, Itakura M: Transgenic expression of IL-10 in pancreatic islet A cells accelerates autoimmune insulitis and diabetes in non-obese diabetic mice. *Int Immunol* 6:1927–1936, 1994
12. Wogensen L, Haung X, Sarvetnick N: Leukocyte extravasation into the pancreatic tissue in transgenic mice expressing interleukin 10 in the islets of Langerhans. *J Exp Med* 178:175–185, 1993
13. Rabinovitch A, Suarez-Pinzon WL, Sorensen O, Bleackley RC, Power RF, Rajotte RV: Combined therapy with interleukin-4 and interleukin-10 inhibits autoimmune diabetes recurrence in syngeneic islet-transplanted nonobese diabetic mice: analysis of cytokine mRNA expression of the graft. *Transplantation* 60:368–374, 1995
14. von Herrath MG, Dyrberg T, Oldstone MB: Oral insulin treatment suppresses virus-induced antigen-specific destruction of beta cells and prevents autoimmune diabetes in transgenic mice. *J Clin Invest* 98:1324–1331
15. Hammond KJL, Poulton LD, Palmisano LJ, Selveira PA, Godfrey DI, Baxter AG: Alpha/beta-T cell receptor (TCR)+CD4-CD8- (NKT) thymocytes prevent insulin-dependent diabetes mellitus in nonobese diabetic (NOD)/Lt mice by the influence of interleukin (IL)-4 and/or IL-10. *J Exp Med* 187:1047–1056, 1998
16. Rapoport MJ, Mor A, Vardi P, Ramot Y, Winker R, Hindi A, Bistritzer T: Decreased secretion of Th2 cytokines precedes up-regulated and delayed secretion of Th1 cytokines in activated peripheral blood mononuclear cells from patients with insulin-dependent diabetes mellitus. *J Autoimmun* 11:635–642, 1998
17. Pennline KJ, Roque-Gaffney E, Monahan M: Recombinant human IL-10 prevents the onset of diabetes in the nonobese diabetic mouse. *J Immunol Immunopathol* 71:169–175, 1994
18. Zheng XX, Steele AW, Hancock WW, Stevens AC, Nickerson PW, Roy-Chaudhury P, Tian Y, Strom TB: A noncytolytic IL-10/Fc fusion protein prevents diabetes, blocks autoimmunity, and promotes suppressor phenomena in NOD mice. *J Immunol* 158:4507–4513, 1997
19. Nitta Y, Tashiro F, Tokui M, Shimada A, Takei I, Tabayashi K, Miyazaki J: Systemic delivery of interleukin 10 by intramuscular injection of expression plasmid DNA prevents autoimmune diabetes in nonobese diabetic mice. *Hum Gene Ther* 10:1701–1707, 1998
20. Hagenbaugh A, Sharma S, Dubinett SM, Wei SH-Y, Aranda R, Cheroutre H, Fowell DJ, Binder S, Tsao B, Locksley RM, Moore KW, Kronenberg M: Altered immune responses in interleukin 10 transgenic mice. *J Exp Med* 185: 2101–2110, 1997
21. Degermann S, Reilly C, Scott B, Ogata L, von Boehmer H, Lo D: On the various manifestations of spontaneous autoimmune diabetes in rodent models. *Eur J Immunol* 24:3155–3160, 1994
22. Lo D, Freedman J, Hesse S, Palmiter RD, Brinster RL, Sherman LA: Peripheral tolerance to an islet cell-specific hemagglutinin transgene affects both CD4+ and CD8+ T cells. *Eur J Immunol* 22:1013–1022, 1992
23. Bergman B, Haskins K: Islet-specific T-cell clones from the NOD mouse

Downloaded from http://diabetesjournals.org/diabetes/article-pdf/48/10/1948/363756/10512358.pdf by guest on 11 October 2024

respond to β-granule antigen. *Diabetes* 43:197–203, 1994
24. Mueller R, Lee M-S, Sawyer SP, Sarvetnick N: Transgenic expression of interleukin 10 in the pancreas renders resistant mice susceptible to low dose streptozotocin-induced diabetes. *J Autoimmun* 9:151–159, 1996
25. Lee M-S, Mueller R, Wicker LS, Peterson LB, Sarvetnick N: IL-10 is necessary and sufficient for autoimmune diabetes in conjunction with NOD MHC homozygosity. *J Exp Med* 183:2663–2668, 1996
26. Lee M-S, Wicker LS, Peterson LB, Sarvetnick N: Pancreatic IL-10 induces diabetes in NOD.B6 *Idd3 Idd10* mice. *Autoimmunity* 26:215–221, 1997
27. Brombacher F, Schafer T, Weissenstein U, Tschopp C, Andersen E, Burki K, Baumann G: IL-2 promoter driven lacZ expression as a monitoring tool for IL-2 expression in primary T cells of transgenic mice. *Int Immunol* 6:189–197, 1994
28. Lo D, Reilly CR, Scott B, Liblau R, McDevitt HO, Burkly L: Antigen-presenting cells in adoptively transferred and spontaneous autoimmune diabetes. *Eur J Immunol* 23:1693–1698, 1993
29. Cua DJ, Coffman RL, Stohlman SA: Exposure to T helper 2 cytokines in vivo before encounter with antigen selects for T helper subsets via alterations in antigen-presenting cell function. *J Immunol* 157:2830–2836, 1996
30. Fiorentino DF, Zlotnik A, Vieira P, Mosmann TR, Howard M, Moore KW, O'Garra A: IL-10 acts on the antigen-presenting cell to inhibit cytokine production by Th1 cells. *J Immunol* 146:3444–3451, 1991
31. Chen W-F, Zlotnik A: IL-10: a novel cytotoxic T cell differentiation factor. *J Immunol* 147:528–534, 1991
32. Jinquan T, Larsen CG, Gesser B, Matsushima K, Thestrup-Pedersen K: Human IL-10 is a chemoattractant for CD8+ T lymphocytes and an inhibitor of IL-8-induced CD4+ T lymphocyte migration. *J Immunol* 15:4545–4551, 1993
33. Balasa B, Davies JD, Lee J, Good A, Yeung BT, Sarvetnick N: IL-10 impacts autoimmune diabetes via a $CD8^+$ T cell pathway circumventing the requirement for $CD4^+$ T and B lymphocytes. *J Immunol* 161:4420–4427, 1998
34. Koppelman B, Neefjes JJ, de Vries JE, de Waal Malefyt R: Interleukin-10 down-regulates MHC class II alpha beta peptide complexes at the plasma membrane of monocytes by affecting arrival and recycling. *Immunity* 7:861–871, 1997

Downloaded from http://diabetesjournals.org/diabetes/article-pdf/48/10/1948/363756/10512358.pdf by guest on 11 October 2024