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Brief Definitive Report

IL-10 Is Necessary and Sufficient for Autoimmune Diabetes in Conjunction with NOD MHC Homozygosity

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Summary

Contrary to expectations based on in vitro experiments, we previously found that pancreatic IL-10 did not inhibit autoimmune diabetes but accelerated it in an MHC-dependent manner. Therefore, the ability of IL-10 to overcome the absence of all non-MHC diabetes susceptibility (*Idd*) alleles was studied in transgenic mice expressing pancreatic IL-10 backcrossed to B10.$H2^{g7}$ congenic mice, which have no *Idd* alleles other than NOD MHC ($H2^{g7}$). IL-10 transgenic backcross 1 (BC1) mice with $H2^{g7/g7}$ haplotype developed clear-cut insulitis and diabetes, but neither transgenic mice with the $H2^{g/b}$ haplotype nor nontransgenic BC1 mice did so. Further implicating IL-10 in autoimmune diabetes, anti-IL-10 antibody treatment inhibited the development of insulitis in NOD mice. These results suggest that IL-10 may be necessary and sufficient for producing autoimmune diabetes in conjunction with NOD MHC homozygosity and that some *Idd* genes may be related to the regulation of IL-10.

IL-10 is produced mainly by Th2 cells, macrophages, and Ly1+ B lymphocytes (1, 2). IL-10 inhibits Th1 cell proliferation and cytokine production in the presence of macrophages by blocking costimulatory activity in vitro (3, 4). These results suggest that IL-10 is a potential agent for the treatment of autoimmune diseases or allograft rejection in vivo. However, experiments using transgenic mice expressing IL-10 in pancreatic β- (Ins-IL-10 mice) or α-cells and their offspring from backcrosses to NOD mice demonstrated that pancreatic IL-10 did not inhibit but, instead, accelerated autoimmune diabetes in an MHC-dependent manner (5–7). Furthermore, results from the backcross experiment suggested that pancreatic IL-10 might overcome the absence of NOD homozygosity at a substantial number of non-MHC diabetes-susceptibility (*Idd*) loci (6). The experiments described in this report were undertaken to determine whether pancreatic IL-10 is able to replace all known non-MHC *Idd* alleles in NOD mice and whether IL-10 is sufficient for the development of autoimmune diabetes in the transgenic mouse model when an appropriate MHC is provided. In addition, the role of IL-10 in the pathogenesis of natural autoimmune diabetes in NOD mice was studied.

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Materials and Methods

Animals. Ins-IL-10 mice of the C57BL/6 background were derived by repeated backcrossing of Ins-IL-10 mice (8) to C57BL/6 mice (B6.Ins-IL-10 mice). Neither insulitis nor diabetes was observed in B6.Ins-IL-10 mice or the original Ins-IL-10 mice of BALB/c background (8). After four backcross generations, these mice were subsequently bred with B10.$H2^{g7}$ congenic mice that have NOD MHC ($H2^{g7}$) but no other NOD-derived Idd alleles (9). IL-10-transgenic F1 mice were backcrossed to B10.$H2^{g7}$ twice to derive IL-10-positive backcross 1 (BC1) mice that were homozygous for the NOD MHC but without other NOD-derived *Idd* alleles: BC1 [F1(B6.Ins-IL-10 × B10.$H2^{g7}$) × B10.$H2^{g7}$] (Fig. 1). In another set of breedings, IL-10-transgenic F1 mice were bred with NOD mice to derive F1 × NOD mice: [F1(B6.Ins-IL-10 × B10.$H2^{g7}$) × NOD]. F1 × NOD mice were heterozygous at all non-MHC *Idd* loci (Fig. 1).

The incidence of diabetes was determined by weekly measurement of blood glucose starting at 5 wk and lasting up to 3 mo of age. Diabetes was defined as a single non fasting blood glucose level over 300 mg/dl or two consecutive measurements over 230 mg/dl in ocular blood samples tested with a Glucometer 3 (Miles Inc., Elkhart, IN).

MHC typing was done by PCR amplification of the I-A_β chain (primers, CTCTTCAGGCTGGGATGCTCCACAT and TGTCTTTTCTGTCACCCTAGAACAG) followed by Msp1 digestion. The amplified material from homozygous ($H2^{g7/g7}$) mice was not cut with Msp1, yielding a single fragment of 282 bp. That from heterozygous ($H2^{g7/b}$) mice contained 282-, 149-, and 133-bp fragments.

mAb Treatment. mAbs were produced by making ascites in

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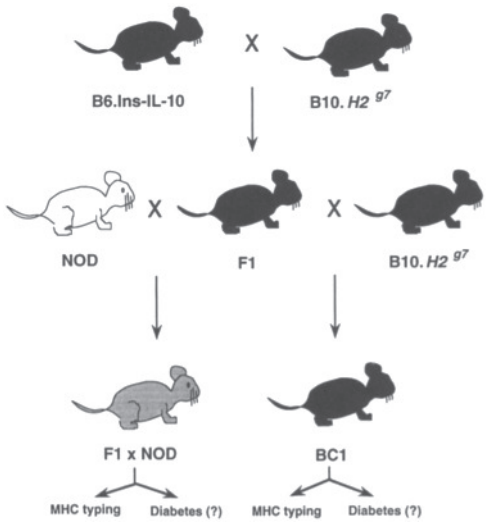

Figure 1. Ins-IL-10 mice of the C57BL/6 background (B6.Ins-IL-10) were derived by repeated backcrossing to C57BL/6 mice. They were bred with B10.$H2^{g7}$ mice, and transgenic F1 mice were backcrossed to B10.$H2^{g7}$ mice to derive transgenic mice that were homozygous for the NOD MHC ($H2^{g7/g7}$) but had no other NOD-derived *Idd* alleles (BC1). Transgenic F1 mice were also bred with NOD mice to derive mice homozygous for the NOD MHC but heterozygous at all non-MHC *Idd* loci (F1 × NOD). The immunogenic significance of the F1 × NOD breeding experiment is similar to that of the B10.$H2^{g7}$ backcross experiment, because all *Idd* alleles of NOD defined in previous backcross studies were either recessive or dominant with very low penetrance.

SCID mice or nude mice with injections of 5×10^6 SXC-1 or JES5-2A5.11 hybridoma cells given 1 wk after pristane (Sigma Chemical Co., St. Louis, MO) injection. Protein was purified from the ascites by using 2-step ammonium sulfate precipitation, and Ig level was quantified with a Bradford protein assay. Anti-IL-10 mAb of the rat IgM (1 μg/injection) or rat IgG class (0.5 μg/injection) was administered to the experimental group three times a week. Control mice were treated with either PBS or rat IgG (Sigma). Scoring of insulitis was done by examining 2 H&E-stained slides taken from different sites of one pancreatic block. If the number of islets was below 10 in both samples, one more slide was prepared from a different site. The degree of insulitis was classified into three categories: no insulitis, periinsulitis with or without minimal lymphocytic infiltration into the islets, and insulitis.

The Chi-square test was employed for statistical analysis.

RNase Protection Assay. Splenic RNA was isolated 4 h after i.p. injection of 350 μg LPS (Sigma) by mechanical homogenization in guanidium thiocyanate solution and acid-phenol extraction. P^{32}-labeled riboprobes were prepared by in vitro transcription of linearized expression vectors harboring bacteriophage promoters. The riboprobes were hybridized to 5 μg of each RNA sample and control tRNA. The protected RNA hybrid and the respective probes were analyzed on a 8% polyacrylamide gel. The signal was quantified using x-ray densitometry and standardized against the band intensity of a control probe (GAPDH).

Immunohistochemistry. Acetone-fixed fresh frozen sections were incubated with anti-B220 (PharMingen, San Diego, CA), anti-CD4 (PharMingen), anti-CD8 (PharMingen) or anti-Mac-1 (Boehringer Mannheim, Indianapolis, IN) Ab. Incubation with an appropriate biotinylated secondary Ab (Vector Laboratories, Burlingame, CA), and then with avidin-biotin-peroxidase complex (Vector Laboratories) followed. After color reaction with diaminobenzidine (Sigma), the sections were counterstained with methyl green.

Results and Discussion

Before 3 mo of age, 8 of 19 IL-10-positive $H2^{g7/g7}$ BC1 mice (42%) became diabetic (Table 1). If fact, most of them developed diabetes before 2 mo of age, with no apparent difference in incidence according to sex. In contrast, neither transgenic BC1 mice with $H2^{g7/b}$ haplotype (n = 14) nor nontransgenic BC1 mice ($H2^{g7/g7}$, n = 16; $H2^{g7/b}$, n = 17) ever developed diabetes during this period. Histopathological examination revealed clearcut insulitis in the pancreata of IL-10-transgenic mice with the $H2^{g7/g7}$ haplotype, regardless of the presence of diabetes (61% of the total islets examined at the time of diabetes onset or at 3 mo of age) (Fig. 2, *A* and *B*). Most pancreatic islets of IL-10-transgenic mice with the $H2^{g7/b}$ haplotype showed periinsulitis characteristic of Ins-IL-10 mice of BALB/c or C57BL/6 background (8) (Fig. 2 *C*), and occasional islets showed minimal lymphocytic infiltration but without islet destruction. Pancreatic islets of nontransgenic mice of both MHC haplotypes were free from periinsulitis or insulitis. To determine whether autoimmunity accounted for the diabetes and insulitis observed here, we transplanted neonatal B10.$H2^{g7}$ pancreata into diabetic or nondiabetic male IL-10-transgenic BC1 mice of the $H2^{g7/g7}$ haplotype. As a result, massive lymphocytic infiltration with abortive islet formation was observed in all pancreatic grafts 3–4 wk after transplantation (n = 3) (Fig. 2 *E*), whereas control grafts placed into the nontransgenic BC1 mice of the $H2^{g7/g7}$ haplotype formed mature islets, with only very rare inflammatory cells (n = 3) (Fig. 2 *D*). The results from the graft experiment and strict dependence of diabetes on NOD MHC homozygosity indicate that autoimmunity was indeed respon-

Table 1. *The Incidence of Diabetes in B10H.2^{g7} BC1 and F1 × NOD Mice Over a 3-mo Observation Period*

B10.$H2^{g7}$ BC1				F1 × NOD			
IL-10 transgenic		Nontransgenic		IL-10 transgenic		Nontransgenic	
$H2^{g7/b}$	$H2^{g7/b}$	$H2^{g7/g7}$	$H2^{g7/b}$	$H2^{g7/g7}$	$H2^{g7/b}$	$H2^{g7/g7}$	$H2^{g7/b}$
8/19 (42%)	0/14	0/16	0/17	7/13 (54%)	0/14	0/12	0/16

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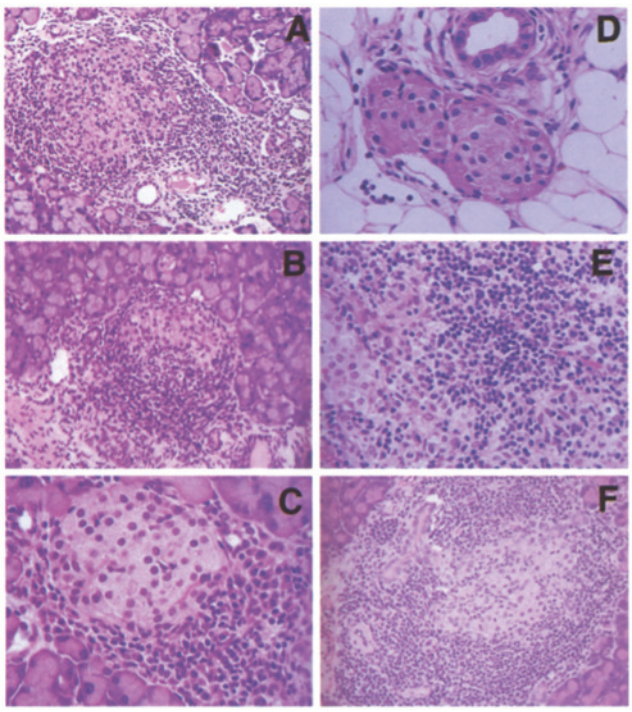

Figure 2. Pancreatic sections from an 8-wk-old diabetic (*A*) and a 3-mo-old nondiabetic (*B*) IL-10-transgenic BC1 mouse of the $H2^{g7/g7}$ haplotype show insulitis with islet destruction (×200). (*C*) The pancreas from a 3-mo-old BC1 mouse of the $H2^{g7/b}$ haplotype shows periinsulitis characteristic of Ins-IL-10 mice but not insulitis (×400). (*D*) A control graft of the neonatal B10.H2g7 pancreas into a male nontransgenic B10.$H2^{g7/g7}$ BC1 mouse shows mature islets with very rare inflammatory cells (×400). (*E*) A graft from the neonatal B10.H2g7 pancreas under the kidney capsule of a male transgenic B10.$H2^{g7/g7}$ BC1 mouse (×400). Massive infiltration of lymphocytes and abortive islet formation were observed 4 wk after transplantation. (*F*) Insulitis with islet destruction was also observed in the pancreas of an 8-wk-old transgenic F1 × NOD mouse of the $H2^{g7/g7}$ haplotype (×200).

sible for the islet destruction in IL-10-transgenic $H2^{g7/g7}$ BC1 mice. Together, these results suggest that pancreatic IL-10 can overcome the absence of all non-MHC *Idd* alleles described in NOD mice. In other words, IL-10 may be sufficient for autoimmune diabetes if NOD MHC homozygosity is provided.

Idd genes revealed in backcross studies should be recessive or dominant with very low penetrance. Thus, the result from the F1 × NOD breeding experiment should tell if pancreatic IL-10 can overcome the absence (actually heterozygosity) of all non-MHC *Idd* alleles described in NOD mice like the B10.$H2^{g7}$ backcross study. 7 of 13 transgenic $H2^{g7/g7}$ F1 × NOD mice (54%) became diabetic before 3 mo of age (Table 1). Yet, transgenic F1 × NOD mice of the $H2^{g7/b}$ haplotype or nontransgenic F1 × NOD mice never became diabetic during this period. Histopathological examination again revealed insulitis with islet destruction in transgenic F1 × NOD mice of the $H2^{g7/g7}$ haplotype (65% of the total islets examined) but not in other three combinations (Fig. 2 *F*). That the incidence of diabetes was less than 100% in both B10.$H2^{g7}$ BC 1 and F1 × NOD mice might be attributed to the fact that only 10% of total islet cell mass would be sufficient for glucose homeostasis and that the observation period was relatively short. Some environmental factor(s) might also be involved, resulting in diabetes in some but not all mice, similar to the NOD parental strain. In contrast to these results, systemic IL-10 administration reportedly decreased the incidence of diabetes in NOD mice (10). This discrepancy might be caused by the different site of cytokine administration (systemic vs. local), which was most clearly demonstrated in case of TGF-β1 (11). Additionally, the time point of IL-10 administration was 10 wk of age in the study involving systemic administration (10), which is much later than that experienced by Ins-IL-10 mice (8).

The mechanism of IL-10-induced autoimmunity is not clearly understood. Transgenic production of IL-10 leads to induction of adhesion molecules and periislet inflammation (8). However, effects attributable to nonspecific inflammation or increased chemotaxis alone cannot account for the IL-10-mediated induction or acceleration of autoimmune diabetes observed in our mice. For instance, pancreatic IFN-γ did not accelerate autoimmune diabetes in our backcross experiments to NOD mice, although IFN-γ transgene expression induced pronounced pancreatic inflammation (data not shown). Likewise, pancreatic expression of TNF-α elicited insulitis but did not accelerate diabetes in NOD background (Flavell, R.A. 1995. Genetic manipulation of cytokine expression in the study of autoimmunity and asthma. The 34th Midwinter Conference of Immunologists, Pacific Grove, CA). Our results also differed from those from an RIP-IL-2 backcross to NOD mice in which IL-2 transgene expression did not overcome the absence of a single copy of the *Idd3-Idd10* segment (12), while IL-10 expression could overcome the absence of all non-MHC *Idd* alleles. Furthermore, in our separate breeding experiment using another strain of congenic mice (NOD.B6PL-Thy1^a-*Idd3-Idd10*), transgenic IL-10 specifically overcame the absence of the *Idd3-Idd10* segment (Lee, M.-S., L.S. Wicker, L.B. Peterson, and N. Sarvetnick, unpublished data). The effect of IL-10 in our model might be related to a shift in the balance between Th1/Th2 responses by IL-10, and a Th2-dominant response may not inhibit the autoimmune destruction of target tissue, contrary to previous belief (13). For instance, anti-B7-1 treatment has been reported to induce Th2-dominant responses in vivo (14), yet the same treatment accelerated autoimmune diabetes in NOD mice (15). However, transgenic expression of IL-4 in the pancreatic β-islet cells did not accelerate autoimmune diabetes in our backcross experiment to NOD mice (data not shown). The discrepancy between IL-4 and IL-10 transgenic NOD mice suggests that the effects of local IL-10 might not be directly related to the Th1/Th2 balance. Thus, IL-10-induced or IL-10-accelerated autoimmune diabetes may entail an unexpected immunological mechanism. Activation of B lymphocytes by IL-10 leading to proliferation and Ig production has been reported (16–18), IL-10 may enhance the antigen-presenting function of B lymphocytes as well re-

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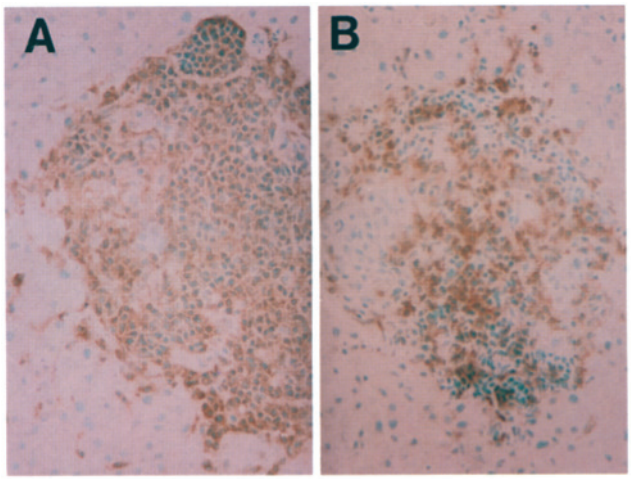

Figure 3. Immunohistochemistry revealed a prominent B lymphocyte infiltrate stained with anti-B220 antibody (*A*) and also a substantial number of $CD4^+$ T lymphocytes (*B*) in pancreatic sections from a transgenic BC1 mouse of the $H2^{g7/g7}$ haplotype (×200). $Mac\text{-}1^+$ macrophages and a small number of $CD8^+$ cells were also present.

sulting in diversification of T cell responses through B lymphocytes functioning as APC as suggested in SLE (19, 20). Increased MHC class II expression on B lymphocytes by IL-10 might be related to the enhanced antigen presentation by B lymphocytes (21). Consistent with this, B lymphocytes stained with anti-B220 antibody were the predominant cells infiltrating the pancreata of transgenic $H2^{g7/g7}$ BC1 and F1 × NOD mice as revealed by immunohistochemistry (Fig. 3 *A*). $CD4^+$ T lymphocytes were the next most abundant cells (Fig. 3 *B*), followed by $Mac\text{-}1^+$ macrophages and a small number of $CD8^+$ T lymphocytes (data not shown).

The foregoing outcomes with our transgenic mice did not indicate a role for IL-10 in the development of natural autoimmune diabetes in NOD mice. To address this issue and to learn if IL-10 is necessary for natural autoimmune diabetes, we administered anti-IL-10 Ab to NOD mice and compared the severity of insulitis between treated groups and control groups. In female NOD mice treated for 6 wk starting at 3–4 wk of age ($n = 12$), the proportion of islets showing insulitis was significantly lower than in control NOD mice treated with PBS or rat IgG ($n = 10$) ($P < 0.001$) (Table 2 A). In another experiment, anti-IL-10 was administered for 3 mo starting when the mice were 3 wk old. Again, virtually no insulitis was observed in their pancreata, whereas insulitis was pronounced in control mice (Table 2 B). This result concurs with the ability of anti-IL-10 to delay the onset of autoimmunity in NZB/W (22) and indicates a larger role for IL-10 in a broad spectrum of autoimmune disorders. These effects might be related to the change in TNF-α level by anti-IL-10 as was suggested in the study involving NZB/W mice; however, regardless of the presence of secondary mediators of IL-10, the results suggest an important role for IL-10 in the pathogenesis of autoimmunity. Yet, our results contrast with a report showing no change in the incidence of diabetes by anti-IL-10 administration to NOD mice (10). However, the NOD mice in that experiment were 10 wk old, a time when effector T lymphocytes are already sensitized to a variety of islet antigens (23, 24). If IL-10 is involved in the pathogenesis of autoimmune diabetes, the critical point would be the initial sensitization phase by APC, not the effector phase mediated by T lymphocytes. If this hypothesis is correct, abrogation of IL-10 after establishment of autoimmunity might not affect the clinical course of diabetes.

The above results suggest that IL-10 may be necessary and sufficient for the development of autoimmune diabetes in conjunction with NOD MHC homozygosity, and also that some uncharacterized non-MHC *Idd* alleles of NOD mice may be related to abnormal production of IL-10 or abnormal sensitivity to IL-10. To examine whether IL-10 regulation differs in NOD and diabetes-resistant mice, IL-10 mRNA levels in their spleens were quantified after LPS administration by using RNase protection assays. Our preliminary results indicated that the ratio of the IL-10 signal to the control (GAPDH) signal was higher in a 5-mo-old female NOD mouse (124%) and a 7-wk-old female NOD mouse (97%) compared to that in a 7-wk-old female BALB/c (23%) and in a 7-wk-old female C57BL/6 mouse (21%). On the other hand, the TNF-α signal normalized for the control signal was highest in the BALB/c mouse (74%), intermediate in the C57BL/6 mouse (47%), and lowest in the 5-mo-old and 7-wk-old NOD mice (both 26%). This preliminary result in mice is consistent with increased IL-10 production in humans with SLE, rheumatoid arthritis or Sjogren's syndrome, suggesting a role for IL-10 regulation not only in autoimmune diabetes but in a broad spectrum of autoimmune diseases (25). The inverse relationship between IL-10 and TNF-α signals might relate to the inhibition of TNF-α expression by IL-10 (22, 26). Moreover, a relationship has been proposed between low TNF-α pro-

Table 2. *Histological Analysis of Islets from Anti-IL-10 and Control-treated NOD Mice*

	A. No. of Islets*			B. No. of Islets‡		
	Normal	Peri-insulitis	Insulitis	Normal	Peri-insulitis	Insulitis
Anti-IL-10	187	14	1	51	15	2
Control	84	57	38	27	44	70

*Effect of anti-IL-10 treatment for 6 wk on insulitis in NOD mice starting at 3–4 wk of age.
IL-10 mAb was administered to the experimental group ($n = 12$), while control mice ($n = 10$) were treated with either PBS or rat IgG. The experimental group had a significantly lower proportion of insulitis and periinsulitis.
‡Effect of anti-IL-10 treatment for 3 mo starting at 3 wk of age. Anti-IL-10 was given to female NOD mice ($n = 5$), while rat IgG or PBS was given to control mice ($n = 8$). Again, anti-IL-10 treatment inhibited the development of insulitis almost completely.

duction itself and the development of autoimmune diseases (27, 28). The susceptibility to autoimmunity in mice with low TNF-α production could be a consequence of their high IL-10 production.

Together, these results strongly suggest a role for IL-10 in the pathogenesis of autoimmune diabetes in both transgenic mice and NOD mice. Previous experiments demonstrating the inhibitory activity of IL-10 were performed in vitro using defined subsets of cells. Immune responses in vivo involve several APC such as dendritic cells, macrophages and B lymphocytes, and their interactions with $CD4^+$ and $CD8^+$ T lymphocytes in which many signals are delivered from one cell to another. Thus, in vivo effects of IL-10 may be different from in vitro findings. B lymphocytes differ from other APCs in that they can concentrate specific proteins with their surface Ig and present even nondominant determinants to T lymphocytes, leading to diversification of T cell responses probably after initial T cell priming to immunodominant determinants by dendritic cells (29). Previous work has demonstrated the existence of low-affinity autoreactive T lymphocytes escaping from thymic censorship (30, 31). Thus, we are tempted to speculate that B lymphocytes stimulated by IL-10 in NOD mice would activate those T lymphocytes reactive to a variety of subdominant or cryptic determinants of self antigens, resulting in diversified T cell responses. A role for B lymphocytes in the development of autoimmune diabetes in NOD mice was suggested in an experiment showing abrogation of diabetes by anti-IgM administration (32). This scenario fits well with the intermolecular and intramolecular spreading of antigenic determinants in autoimmune diabetes and experimental allergic encephalomyelitis (23, 24, 33). Accordingly, we propose that IL-10 can affect interactions between B and T lymphocytes and the resultant diversification of T cell responses, which are critical to the progression to clinical autoimmune diseases.

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References

1. Malefyt, R.D.W., J. Haanen, H. Spits, M.-G. Roncarolo, A.T. Velde, C. Figdor, K. Johnson, R. Kastelein, H. Yssel, and J.E. de Vris. 1991. 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.
2. Fiorentino, D.F., A. Zlotnik, P. Vieira, T.R. Mosmann, M. Howard, K.W. Moore, and A. O'Garra. 1991. IL-10 acts on the antigen-presenting cell to inhibit cytokine production by Th1 cells. *J. Immunol.* 146:3444–3451.
3. Ding, L., and E.M. Shevach. 1992. IL-10 inhibits mitogen-induced T cell proliferation by selectively inhibiting macrophage costimulatory function. *J. Immunol.* 148:3133–3139.
4. Ding, L., P.S. Linsley, L.-Y. Huang, R.N. Germain, and E.M. Shevach. 1993. IL-10 inhibits macrophage costimulatory activity by selectively inhibiting the up-regulation of B7 expression. *J. Immunol.* 151:1224–1234.
5. Lee, M.-S., L. Wogensen, J. Shizuru, M.B.A. Oldstone, and N. Sarvetnick. 1994. Pancreatic islet production of murine interleukin 10 does not inhibit immune-mediated tissue destruction. *J. Clin. Invest.* 93:1332–1338.
6. Wogensen, L., M.-S. Lee, and N. Sarvetnick. 1994. Production of interleukin 10 by islet cells accelerates immune-mediated destruction of b cells in nonobese diabetic mice. *J. Exp. Med.* 179:1379–1384.
7. Moritani, M., K. Yoshimoto, F. Tashiro, C. Hashimoto, J. Miyazaki, S. Ii, E. Kudo, H. Iwahana, Y. Hayashi, T. Sano, and M. Itakura. 1994. 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.
8. Wogensen, L., X. Huang, and N. Sarvetnick. 1993. Leukocyte extravasation into the pancreatic tissue in transgenic mice expressing interleukin-10 in the islets of Langerhans. *J. Exp. Med.* 178:175–185.
9. Ghosh, S., S.M. Palmer, N.R. Rodrigues, H.J. Cordell, C.M. Hearne, R.J. Cornall, J.-B. Prins, P. McShane, G.M. Lathrop, L.B. Peterson et al. 1993. Polygenic control of autoimmune diabetes in nonobese diabetic mice. *Nat. Genet.* 4:404–409.
10. Pennline, K.J., E. Roque-Gaffney, and M. Monahan. 1994. Recombinant human IL-10 prevents the onset of diabetes in the nonobese diabetic mouse. *Clin. Immunol. Immunopathol.* 71:169–175.
11. Wahl, S.M. 1994. Transforming growth factor β: the good, the bad, and the ugly. *J. Exp. Med.* 180:1587–1590.
12. Allison, J., P. McClive, L. Oxbrow, A. Baxter, G. Morahan, and J.F.A.P. Miller. 1994. Genetic requirements for acceleration of diabetes in non-obese diabetic mice expressing interleukin-2 in islet β-cells. *Eur. J. Immunol.* 24:2535–2541.
13. Katz, J.D., C. Benoist, and D. Mathis. 1995. T helper cell

subset in insulin-dependent diabetes. *Science (Wash. DC).* 268:1185–1188.

14. Kuchroo, V.K., M.P. Das, J.A. Brown, A.M. Ranger, S.S. Zamvil, R.A. Sobel, H.L. Weiner, N. Nabavi, and L.H. Glimcher. 1995. B7-1 and B7-2 costimulatory molecules activate differentially the Th1/Th2 development pathways: application to autoimmune disease therapy. *Cell.* 80:707–718.
15. Lenschow, D.J., S.C. Ho, H. Sattar, L. Rhee, G. Gray, N. Nabavi, K.C. Herold, and J.A. Bluestone. 1995. Differential effects of anti-B7-1 and anti-B7-2 monoclonal antibody treatment on the development of diabetes in the nonobese diabetic mouse. *J. Exp. Med.* 181:1145–1155.
16. Rousset, F., E. Garcia, T. Defrance, C. Peronne, N. Vezzio, D.-H. Hsu, R. Kastelein, K.W. Moore, and J. Banchereau. 1992. Interleukin 10 is a potent growth and differentiation factor for activated human B lymphocytes. *Proc. Natl. Acad. Sci. USA.* 89:1890–1893.
17. Defrance, T., B. Vanbervliet, F. Briere, I. Durand, F. Rousset, and J. Banchereau. 1992. Interleukin 10 and transforming growth factor β cooperate to induce anti-CD40-activated naive human B cells to secrete immunoglobulin A. *J. Exp. Med.* 175:671–682.
18. Llorente, L., W. Zou, Y. Levy, Y. Richaud-Patin, J. Widjenes, J. Alcocer-Varela, B. Morel-Fourrier, J.-C. Brouet, D. Alarcon-Segovia, G. Pierre, and D. Emilie. 1995. Role of interleukin 10 in the B lymphocyte hyperactivity and autoantibody production of human systemic lupus erythematosus. *J. Exp. Med.* 181:839–844.
19. Fatenejad, S., M.J. Mamula, and J. Craft. 1993. Role of intermolecular/intrastructural B- and T-cell determinants in the diversification of autoantibodies to ribonucleoprotein particles. *Proc. Natl. Acad. Sci. USA.* 90:12010–12014.
20. Mamula, M.J., S. Fataenejad, and J. Craft. 1994. B cells process and present lupus autoantigens that initiate autoimmune T cell responses. *J. Immunol.* 152:1453–1461.
21. Go, N.F., B.E. Castle, R. Barrett, R. Kastelein, W. Dang, T.R. Mosmann, K.W. Moore, and M. Howard. 1990. Interleukin-10, a novel B cell stimulatory factor: Unresponsiveness of X chromosome-linked immunodeficiency B cells. *J. Exp. Med.* 172:1625–1631.
22. Ishida, H., T. Muchamuel, S. Sakaguchi, S. Andrade, S. Menon, and M. Howard. 1994. Continuous administration of anti-interleukin 10 antibodies delays onset of autoimmunity in NZB/W F1 mice. *J. Exp. Med.* 179:305–310.
23. Kaufman, D.L., M. Clare-Salzler, J. Tian, T. Forsthuber, G.S.P. Ting, P. Robinson, M.A. Atkinson, E.E. Sercarz, A.J. Tobin, and P.V. Lehmann. 1993. Spontaneous loss of T-cell tolerance to glutamic acid decarboxylase in murine insulin-dependent diabetes. *Nature (Lond.).* 366:69–72.
24. Tisch, R., X.-D. Yang, S.M. Singer, R.S. Libiau, L. Fugger, and H.O. McDevitt. 1993. Immune response to glutamic acid decarboxylase correlates with insulitis in non-obese diabetic mice. *Nature (Lond.).* 366:72–75.
25. Llorente, L., Y. Richaud-Patin, R. Fior, J. Alcocer-Varela, J. Wijdenes, B.M. Fourrier, P. Galanaud, and D. Emilie. 1994. In vivo production of interleukin-10 by non-T cells in rheumatoid arthritis, Sjogren's syndrome, and systemic lupus erythematosus. *Arthritis Rheum.* 37:1647–1655.
26. Howard, M., T. Muchamuel, S. Andrade, and S. Menon. 1993. Interleukin-10 protects mice from lethal endotoxemia. *J. Exp. Med.* 177:1205–1208.
27. Jacob, C.O., S. Aiso, S.A. Michie, H.O. McDevitt, and H. Acha-Orbea. 1990. Prevention of diabetes in nonobese diabetic mice by tumor necrosis factor (TNF): Similarities between TNF-α and interleukin 1. *Proc. Natl. Acad. Sci. USA.* 87:968–972.
28. Satoh, J., H. Seino, T. Abo, S. Tanaka, S. Shitani, S. Ohta, K. Tamura, T. Sawai, T. Nobunaga, T. Oteki et al. 1989. Recombinant human tumor necrosis factor α suppresses autoimmune diabetes in nonobese diabetic mice. *J. Clin. Invest.* 84: 1345–1348.
29. Mamula, M.J., and C.A. Janeway. 1993. Do B cells drive the diversification of immune responses? *Immunol. Today.* 14:151–152.
30. Heath, W.R., J. Allison, M.W. Hoffmann, G. Schonrich, G. Hammerling, B. Arnold, and J.F.A.P. Miller. 1992. Autoimmune diabetes as a consequence of locally produced interleukin-2. *Nature (Lond.).* 359:547–549.
31. Saruhan-Direskeneli, G., F. Weber, E. Meinl, M. Pette, G. Giegerich, A. Hinkkanen, J.T. Epplen, R. Hohlfeld, and H. Wekerle. 1993. Human T cell autoimmunity against myelin basic protein:CD4+ cells recognizing epitopes of the T cell receptor b chain from a myelin basic protein-specific T cell clone. *Eur. J. Immunol.* 23:530–536.
32. Forsgren, S., A. Andersson, V. Hillorn, A. Soderstrom, and D. Holmberg. 1991. Immunoglobulin-mediated prevention of autoimmune diabetes in the non-obese diabetic (NOD) mouse. *Scand. J. Immunol.* 34:445–451.
33. Lehmann, P.V., T. Forsthuber, A. Miller, and E.E. Servart. 1992. Spreading of T-cell autoimmunity to cryptic determinants of an autoantigen. *Nature (Lond.).* 358:155–157.