# Neutralization of Interleukin-16 Protects Nonobese Diabetic Mice From Autoimmune Type 1 Diabetes by a CCL4-Dependent Mechanism

**Craig Meagher,[1,2] Josh Beilke,[3] Guillermo Arreaza,[2] Qing-Sheng Mi,[2] Wei Chen,[2] Konstantin Salojin,[2] Noah Horst,[3] William W. Cruikshank,[4] and Terry L. Delovitch[1,2]**

**OBJECTIVE—**The progressive infiltration of pancreatic islets by lymphocytes is mandatory for development of autoimmune type 1 diabetes. This inflammatory process is mediated by several mediators that are potential therapeutic targets to arrest development of type 1 diabetes. In this study, we investigate the role of one of these mediators, interleukin-16 (IL-16), in the pathogenesis of type 1 diabetes in NOD mice.

**RESEARCH DESIGN AND METHODS—**At different stages of progression of type 1 diabetes, we characterized IL-16 in islets using GEArray technology and immunoblot analysis and also quantitated IL-16 activity in cell migration assays. IL-16 expression was localized in islets by immunofluorescence and confocal imaging. In vivo neutralization studies were performed to assess the role of IL-16 in the pathogenesis of type 1 diabetes.

**RESULTS—**The increased expression of IL-16 in islets correlated with the development of invasive insulitis. IL-16 immunoreactivity was found in islet infiltrating T-cells, B-cells, NK-cells, and dendritic cells, and within an insulitic lesion, IL-16 was derived from infiltrating cells. $CD4^+$ and $CD8^+$ T-cells as well as $B220^+$ B-cells were identified as sources of secreted IL-16. Blockade of IL-16 in vivo protected against type 1 diabetes by interfering with recruitment of $CD4^+$ T-cells to the pancreas, and this protection required the activity of the chemokine CCL4.

**CONCLUSIONS—**IL-16 production by leukocytes in islets augments the severity of insulitis during the onset of type 1 diabetes. IL-16 and CCL4 appear to function as counterregulatory proteins during disease development. Neutralization of IL-16 may represent a novel therapy for the prevention of type 1 diabetes. ***Diabetes*** **59:2862–2871, 2010**

Insulitis and type 1 diabetes are mediated by the regulated homing of leukocytes to pancreatic islets by chemoattractants (1–3). Unlike chemokines that bind chemokine receptors, the interleukin-16 (IL-16) cytokine binds to CD4 and selectively recruits $CD4^+$ T helper one (Th1) cells due to the functional relationship between CD4 and the CCR5 and CXCR3 chemokine receptors (4,5). Mature IL-16 is secreted after caspase-3 mediated cleavage of either neuronal IL-16 or precursor protein (pro-IL-16) (6–13). IL-16 also regulates T-cell activation, CD25 and major histocompatibility complex class II expression, IL-2 production (14), antigen-induced upregulation of CD95 expression (14), and chemokine-induced chemoattraction (15,16). Binding of IL-16 to CD4 selectively desensitizes CCL4/CCR5-induced chemotaxis, and pretreatment of T-cells with CCL4 blocks chemotaxis triggered by IL-16 binding to CD4 (15). CD4 and CCR5 may therefore share a unique relationship mediated by either IL-16 and/or CCL4 binding, and IL-16 and CCL4 may cross-regulate each other's activity. Thus, IL-16 is an immunomodulatory and proinflammatory cytokine that influences the recruitment and activation of $CD4^+$ T-cells in vivo.

Given that $CD4^+$ T-cells mediate the development of type 1 diabetes, we examined whether IL-16 influences $CD4^+$ T-cell recruitment to pancreatic islets. We show that IL-16 potentiates insulitis and type 1 diabetes in NOD mice, and that anti-IL-16 antibody therapy protects against type 1 diabetes even when administered at the mid-late stages of disease progression. This protection results from reduced trafficking of $CD4^+$ T-cells to islet lesions and requires CCL4 activity. Thus, IL-16 may represent a novel therapeutic target for the prevention of type 1 diabetes.

## RESEARCH DESIGN AND METHODS

NOD/Del, NOD.*Scid*, and NOD.RAGKO mice were housed at the Robarts Research Institute (London, ON, Canada). C57BL/6J mice were purchased from The Jackson Laboratory (Bar Harbor, ME). The NOD-BDC2.5.Thy1.1 mice were a kind gift of the Bluestone laboratory at the University of California San Francisco. Mice were maintained in a specific pathogen-free barrier facility according to Institutional guidelines.

**Reagents.** The 14.1 (mouse IgG2a[κ]) and 17.1 (mouse IgG1) anti-IL-16 monoclonal antibodies (mAbs), which react with mouse and human IL-16, were purified from ascites on a Protein A affinity column (15).

**GEArray technology.** Changes in gene expression were analyzed using GEArray technology according to the manufacturer's directions (SuperArray Biosciences). A detailed protocol is presented in the supplementary Material and Methods, available in an online appendix at http://diabetes.diabetesjournals.org/cgi/content/full/db09-0131/DC1.

**In vivo treatment of mice.** NOD mice were injected intraperitoneally 3 times weekly with the 14.1 anti-IL-16 neutralizing mAb as follows: either 0.1 mg from 3–16 weeks of age or 0.2 mg from 3 to 7, 9 to 16, or 17 to 22 weeks of age. Control mice received isotype-matched mouse IgG2a(κ) (UPC 10, 200 μg, Sigma) (17). To determine whether CCL4 mediates anti-IL-16 induced protection from type 1 diabetes, 0.2 mg of anti-IL-16 (14.1) and 0.1 mg of anti-mouse CCL4 mAb (R&D Systems) were coadministered 3 times weekly from 9 to 16 weeks of age. Control mice received isotype-matched control IgG2a(κ) (UPC 10) and rat IgG (Sigma). A blood glucose level (BGL) of ≥11.1 mmol/l on two consecutive readings indicated the onset of type 1 diabetes.

**Histopathology.** Pancreata were fixed in 10% buffered formalin, embedded in paraffin, and sectioned at 7 μm intervals. The incidence and severity of

From the [1]Department of Microbiology and Immunology, University of Western Ontario, London, Ontario, Canada; the [2]Laboratory of Autoimmune Diabetes, Robarts Research Institute, London, Ontario, Canada; the [3]Department of Immunology, University of California, San Francisco, California; and [4]The Pulmonary Center, Boston University School of Medicine, Boston, Massachusetts.

Corresponding author: Terry L. Delovitch, del@robarts.ca.

Received 28 January 2009 and accepted 27 July 2010. Published ahead of print at http://diabetes.diabetesjournals.org on 6 August 2010. DOI: 10.2337/db09-0131.

C.M. is currently affiliated with Dendreon Corporation, Seattle, Washington.

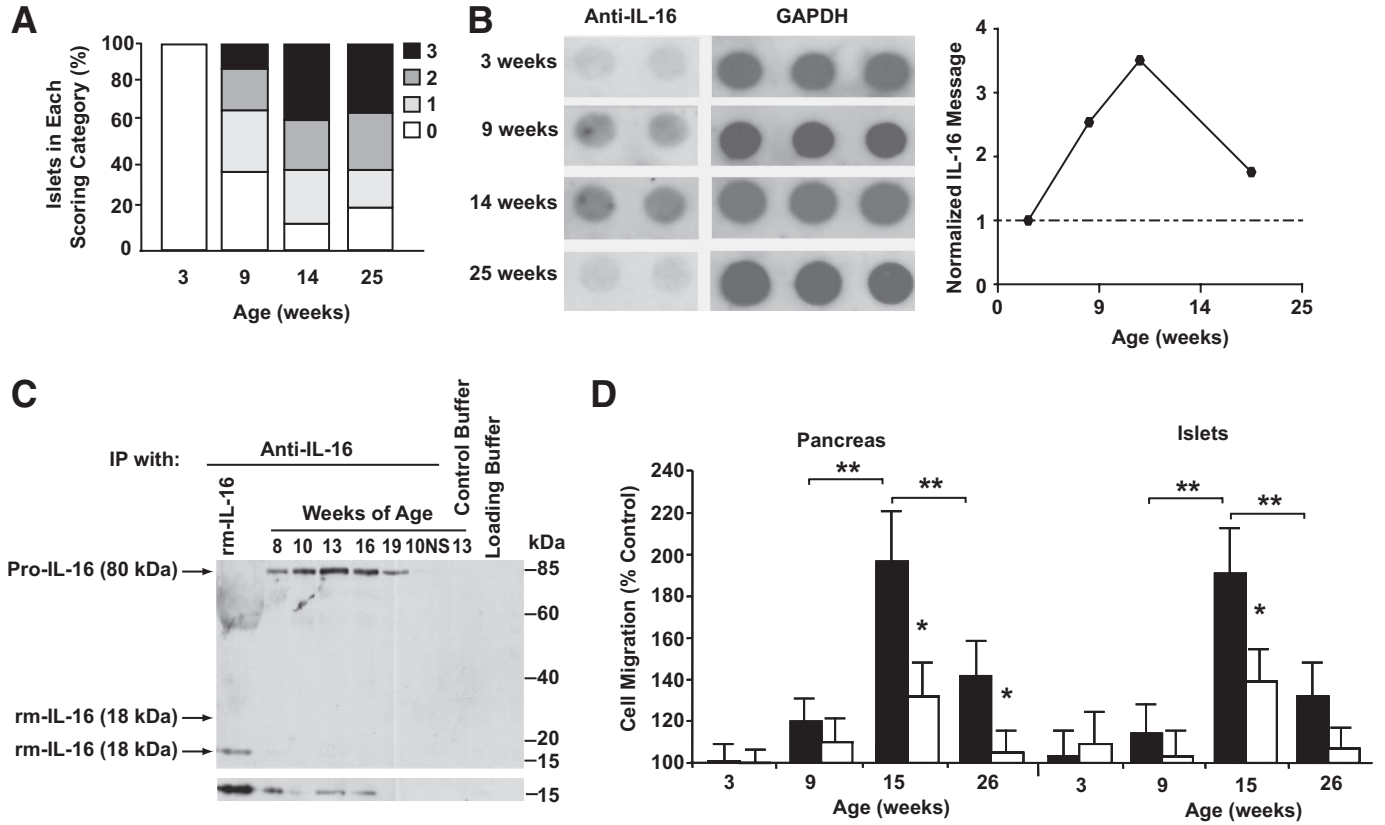


**FIG. 1. Quantitation of IL-16 mRNA and chemotactic activity in the pancreas and islets. *A*: Severity of insulitis in female NOD mice at the indicated ages. *B*: GEArray analysis of intra-islet IL-16 mRNA in total islet RNA isolated at the indicated ages. The relative amount of IL-16 transcript at each age was estimated by comparing its average signal intensity with the average signal derived from glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and normalized to 3 weeks of age. One of two representative experiments is shown. *C*: Immunoprecipitation of IL-16 isoforms in pancreata from NOD and NOD.Scid (NS) mice. Lysate aliquots were immunoprecipitated as described in RESEARCH DESIGN AND METHODS. All blots were initially developed by electrochemiluminescence. Visualization of mature IL-16 was enhanced by stripping, reblotting, and detecting with the more sensitive ECL-Plus reagent (*lower panel*). Stripping and reblotting with nonspecific isotype-matched biotinylated control failed to detect IL-16. Results are from one of three representative and reproducible experiments. *D*: Production of bioactive IL-16 in the pancreas and islets. IL-16 chemoattractant activity was analyzed at the indicated ages using a modified Boyden chamber assay. For the indicated ages, purified splenic T-cells ($5 \times 10^6$) were incubated with either immunoaffinity-isolated IL-16 samples from islets and pancreas (black bars) or a neutralizing anti-IL-16 mAb (5 μg/ml; white bars). *Indicates IL-16 induced chemotaxis was significantly neutralized by anti-IL-16, $P < 0.05$. **Denotes significant IL-16-induced chemotaxis at the indicated ages, $P < 0.05$. Similar results were obtained in two independent and reproducible experiments.**

insulitis were examined after hematoxylin–eosin (H&E) staining. At least 10 nonadjacent sections were analyzed per pancreas, and average percentages of insulitis were determined from ≥100 islets per treatment group.

**Adoptive T-cell transfer.** NOD.*Scid* mice (5–7 weeks old, $n = 8$/group) were injected intraperitoneally with spleen T-cells ($4 \times 10^6$ per mouse) isolated at 16 weeks of age from NOD mice treated 3 times weekly from 9 to 16 weeks of age with 200 μg of anti-IL-16 (14.1) or isotype control IgG2a(κ).

**Statistical analysis.** Significant differences were determined using the Student *t* test for unpaired samples, the Mantel-Haenszel log-rank test, and the Mann-Whitney nonparametric test where indicated. Statistical comparisons were performed using GraphPad Prism (www.graphpad.com; San Diego, CA). A probability value of $P \leq 0.05$ was considered significant.

A supplementary Research Design and Methods section is available in the online appendix.

## RESULTS

**IL-16 expression during development of insulitis.** In our NOD female mice, nondestructive peri-insulitis develops from 6 to 10 weeks of age and is followed by an invasive and destructive insulitis in 15–25-week-old mice (Fig. 1*A*). Onset of diabetes is observed as early as 13 weeks of age, progressing to a cumulative disease incidence of 80–90% by 30 weeks of age. Cytokine GEArray analyses of changes in islet cytokine expression revealed that, relative to 3-week-old mice, IL-16 mRNA was increased >2-fold in 9-week-old mice and reached a maximum by 15 weeks of age (Fig. 1*B*). IL-16 mRNA expression decreased between 15 and 25 weeks of age, even though insulitis persists during this period. This may reflect a reduced number of total islet infiltrating leukocytes resulting from the destruction of a majority of islets by this age.

The above-described analyses of intra-islet IL-16 mRNA detected expression of all IL-16 isoforms. We therefore characterized the IL-16 protein isoforms in the pancreas and islets at progressive stages of insulitis, noting that only the mature IL-16 isoform is secreted and chemotactic for $CD4^+$ cells. Initially, IL-16 was immunoprecipitated from islet and pancreas lysates between 3 and 19 weeks of age. Intrapancreatic pro-IL-16 (80 kDa) expression increased during this timeframe and peaked near 16 weeks of age (Fig. 1*B*). Similarly, mature IL-16 (18 kDa) was detected between 8 and 16 weeks of age. Because a very low level of 80 kDa pro-IL-16 was detected in the pancreas of NOD.*Scid* mice (Fig. 1*C*), IL-16 is expressed in the pancreas in the absence of insulitis and increases with disease progression. To more accurately quantitate the levels of mature IL-16, IL-16 was enriched from pancreas and islet lysates by immunoaffinity chromatography and assayed for its ability to induce $CD4^+$ T-cell chemotaxis. This enabled

the quantitation of only mature IL-16 in contrast to other approaches, e.g., ELISA, that due to the limited specificities of commercially available antibodies do not differentiate among the various IL-16 isoforms. For each lysate, IL-16 was enriched using an equivalent amount of total protein. IL-16 chemotactic activity in the islets and pancreas increased significantly among 3 and 15 weeks of age, with the largest increase detected between 9 and 15 weeks (Fig. 1*D*). Note that IL-16 activity was reduced appreciably by 26 weeks of age, indicative of the destruction of the majority of islets at this time of islet isolation. The specificity of IL-16–induced T-cell chemotaxis was demonstrated by the capacity of the 14.1 neutralizing anti-IL-16 mAb to abrogate this chemotactic response. Thus, levels of mature IL-16 in islets increase in an age-dependent manner, suggesting that mature IL-16 is associated with proinflammatory responses against islets.

**In situ localization of IL-16 in islets.** To examine which cells produce IL-16 in an insulitic lesion, immunohistochemistry and confocal imaging was used. Because IL-16 is synthesized as pro-IL-16 and cleaved only by activated caspase-3 to produce mature IL-16 (10), cells secreting IL-16 were identified by triple immunostaining for IL-16, active caspase-3, and various cell surface markers of immune cells. IL-16 immunoreactivity was found in a majority of mononuclear cells in insulitic lesions, including Thy1$^+$ T-cells, B220$^+$ B-cells, CD4$^+$ and CD8$^+$ T-cells, p46$^+$ NK-cells, and CD11c$^+$ dendritic cells (Fig. 2 and supplementary Fig. 1, available in an online appendix). For all experiments, the appropriate isotype controls were used to rule out nonspecific staining (supplementary Fig. 2*A*, available in an online appendix). As determined by colocalized staining for activated caspase-3, Thy1$^+$ T-cells, B220$^+$ B-cells, and both CD4$^+$ and CD8$^+$ T-cells produce mature IL-16. Colocalization of IL-16 and activated caspase-3 occurred mainly in a polarized manner. While many lymphocytes constitutively express pro-IL-16, only a low frequency of lymphocytes stained for activated caspase-3, in support of our immunoprecipitation studies that displayed a low level of mature IL-16 in the pancreas. Because IL-16 expression was not detected in insulin-producing islet β-cells, IL-16 production appears to be restricted to immune cells in an islet lesion in support of our finding that IL-16 is not expressed in islets from NOD.RAGKO mice (supplementary Fig. 2*B*). Thus, in an insulitic lesion, mature IL-16 is secreted by several types of immune cells, and this may enable these cells to recruit additional CD4$^+$ T-cells to an islet lesion and augment an ongoing inflammatory response.

**Neutralizing anti-IL-16 mAb prevents insulitis and type 1 diabetes.** Having determined that intra-islet expression of mature IL-16 increases with the severity of insulitis, we next examined whether blockade of endogenous IL-16 can prevent type 1 diabetes. NOD mice were treated with a neutralizing anti-IL-16 mAb or isotype-matched control antibody from 3 to 16 weeks of age when intrapancreatic levels of IL-16 are increased. This treatment reduced the overall severity of insulitis and prevented the onset of destructive insulitis as insulin expression was preserved (Fig. 3*A*). In comparison to 33-week-old NOD mice treated with isotype control antibody (group 1), the percentage of islets (14%) displaying severe insulitis (insulitis score = 3) in anti-IL-16 treated mice (group 2) was significantly less than that observed (53%) in control mice. Anti-IL-16 treated mice still possessed 52% normal healthy islets (insulitis score = 0), whereas only 20% of islets were healthy in the control-treated mice. In addition, the incidence of type 1 diabetes was reduced from 88% (16/18 mice) in control-treated mice to 44% (8/18 mice; $P = 0.004$) in anti-IL-16 treated mice at 33 weeks of age (Fig. 3*B*). These data show that neutralization of IL-16 protects against insulitis and type 1 diabetes.

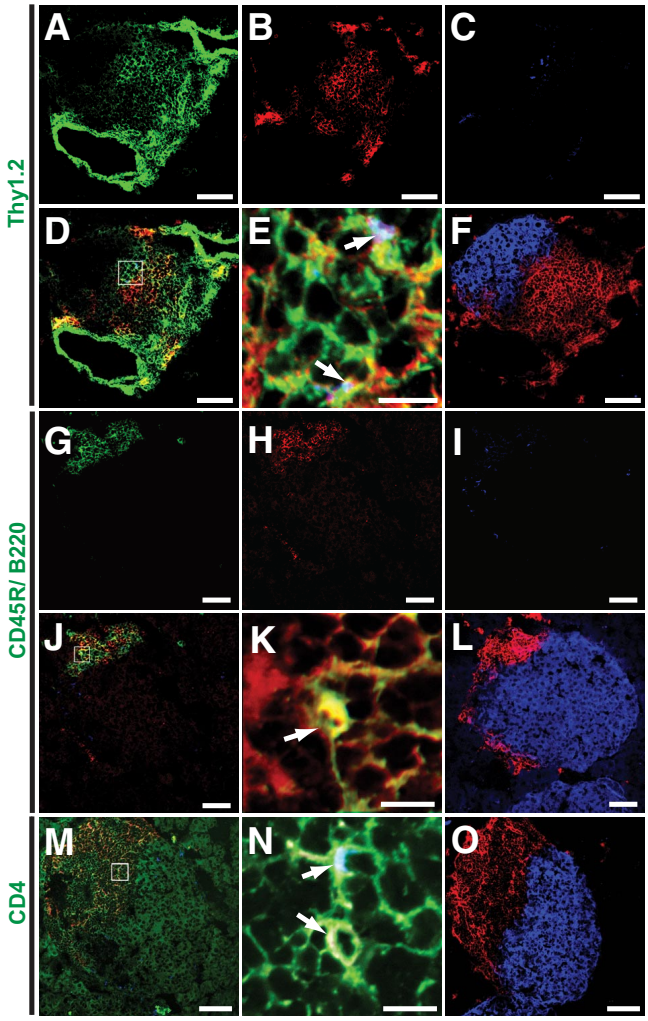


**FIG. 2. In situ localization of IL-16 to lymphocytes in islets of 10-week-old NOD mice. Pancreas sections were stained with anti-IL-16 (red; all panels), anti-activated caspase-3 (blue; *C–E*, *I*, *J*, *M*, and *N*), anti-insulin (blue; *F*, *L*, and *O*), and various cell surface markers in green (Thy1.2 in *A*, *D*, and *E*; B220 in *G*, *J*, and *K*; CD4 in *M* and *N*). Magnified images of areas indicated by the white boxes in merged images (*D*, *J*, and *M*) are presented in *E*, *K*, and *N*. Colocalized signals of IL-16, activated caspase-3, and Thy1.2 (*E*) or B220 (*K*) or CD4 (*N*) are denoted by arrows. Adjacent serial sections stained for IL-16 and insulin are shown in *F*, *L*, and *O*. Scale bars represent 50 μm for all images except for the magnified images (*E*, *K*, *N*), where the scale bar represents 10 μm. (A high-quality digital representation of this figure is available in the online issue.)**

Because intrapancreatic IL-16 expression peaks between 9 and 16 weeks of age, we reasoned that neutralization of IL-16 may prevent type 1 diabetes more effectively during this time than at a younger age when intra-islet levels of mature IL-16 are low. To test this possibility, anti-IL-16 or control antibody was administered during either 3–7 weeks of age, 9–16 weeks of age, or after the onset of invasive insulitis. At 33 weeks of age, a similar incidence of type 1 diabetes was initially observed

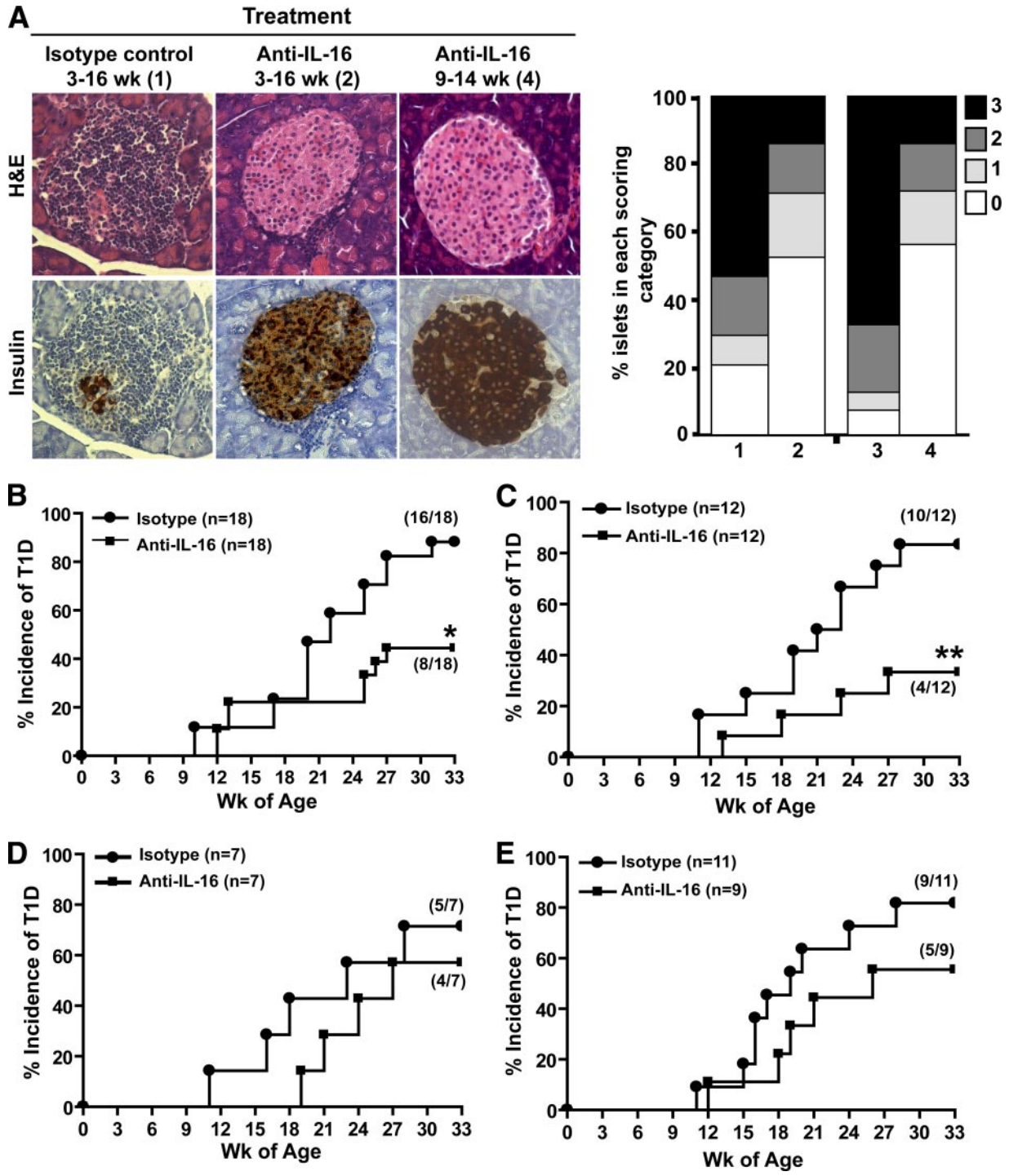


**FIG. 3. Neutralization of IL-16 prevents destructive insulitis and type 1 diabetes in NOD mice in a time- and dose-dependent manner. *A*: Representative H&E- and insulin-stained pancreas sections from NOD mice at 33 weeks of age previously treated three times weekly from 3 to 16 weeks of age with 100 μg of isotype control (1) or anti-IL-16 (2), or from 9 to 16 weeks of age with 200 μg isotype control (3) (note: H&E images are not shown) or anti-IL-16 (4). At 33 weeks, the severity of insulitis was similar for mice treated with isotype control antibody from 9 to 16 (3) or 3 to 16 weeks of age (1). Bar graphs represent the percent of islets in each insulitis scoring category for each denoted treatment group. A representative islet of the most prevalent insulitis score observed for each treatment group at 33 weeks of age is presented. More than 100 islets were analyzed for each treatment group. NOD mice treated three times weekly with anti-IL-16 from either 3 to 16 (*B*) ($P = 0.005$) or 9 to 16 weeks of age (*C*) ($P = 0.015$), but not from 3 to 7 (*D*) ($P = 0.45$) or 17 to 22 weeks of age (*E*) ($P = 0.19$), are protected against type 1 diabetes compared with control-treated mice. (A high-quality digital representation of this figure is available in the online issue.)**

for NOD mice treated between 3 and 14 weeks of age with either 100 or 200 μg of anti-IL-16 mAb per injection (8/18 = 44%, Fig. 3*B*; and 3/8 = 37.5%, data not shown), respectively. Thus, a dose range of 100–200 μg per injection is most effective for neutralization of IL-16. As short treatment regimens were used, mice were injected with a two-fold higher dose of antibody than that used for longer treatments (3–16 weeks of age). Compared with control mice treated from 9 to 16 weeks of age (group 3; H&E images not shown), anti-IL-16 treatment prevented the development of destructive insulitis (group 4) with protection being equally effective to that in mice treated with

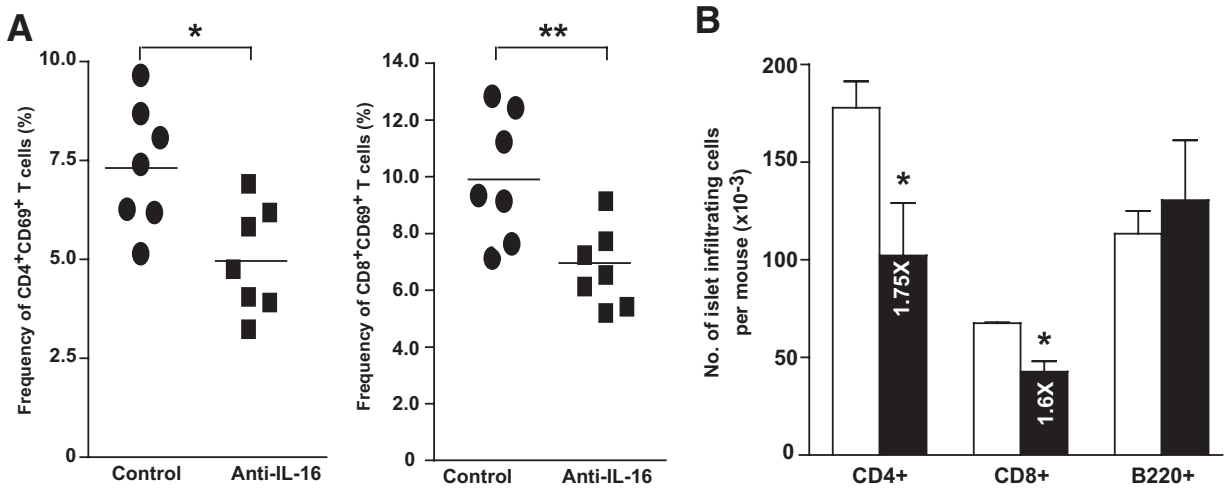


**FIG. 4. Anti-IL-16 protective therapy inhibits T-cell activation in the pancreas and islet infiltration by T-cells. *A*: Independent groups of NOD mice were treated with anti-IL-16 (■) or control antibody (●) 3 times per week from 9 to 16 weeks of age, at which time PLN-derived T-cells were pooled from seven groups of three mice immediately after treatment. Surface CD69 expression was then determined using flow cytometry (*$P \leq 0.003$; **$P < 0.02$). *B*: NOD mice ($n = 6$–8/group) were treated as above and the frequencies of islet-infiltrating lymphocytes were determined by flow cytometry, allowing for lymphocyte cell numbers per pancreas to be calculated. The numbers of islet infiltrating lymphocytes per mouse obtained in three experiments were [(2.5, 2.8, 3.1) × $10^5$] for anti-IL-16 and [(3.4, 3.8, 2.9) × $10^5$] for control-treated mice. Numbers in the bars indicate the fold change for the particular T-cell subset (*$P < 0.03$).**

anti-IL-16 from 3 to 16 weeks of age (group 2). Importantly, the incidence of type 1 diabetes was reduced ($P = 0.015$) by 50% in the 9–16-week-old anti-IL-16 treated mice (4/12 mice = 33%) relative to control-treated mice (10/12 mice = 83%) (Fig. 3*C*). In contrast, anti-IL-16 did not prevent type 1 diabetes ($P = 0.451$) when administered between 3 and 7 weeks of age (Fig. 3*D*). In addition, anti-IL-16 treatment from 17–22 weeks of age (after disease onset when severe insulitis predominates) was ineffective ($P = 0.194$) at protecting against type 1 diabetes (Fig. 3*E*). Note that the median survival time of 26 weeks in anti-IL-16 treated mice exceeded that of 19 weeks in control-treated mice. Therefore, IL-16 activity is not critical for the initiation of disease but is important for the transition from noninvasive to destructive insulitis. Neutralization of IL-16 during this transition elicits maximum protection against type 1 diabetes.

**Anti-IL-16 treatment decreases T-cell activation in the pancreatic lymph nodes and diminishes T-cell recruitment to islets.** Because IL-16 recruits $CD4^+$ T-cells to sites of inflammation and mature IL-16 levels increase more than threefold in the islets and pancreas of 3–15-week-old NOD mice, we analyzed whether anti-IL-16 treatment from 9 to 16 weeks of age protects from type 1 diabetes by blocking the recruitment to and activation of $CD4^+$ T-cells in the pancreas. Consistent with our result that anti-IL-16 therapy from 9–16 weeks of age prevents invasive insulitis and type 1 diabetes, CD69 expression on $CD4^+$ ($P = 0.010$) and $CD8^+$ T-cells ($P = 0.012$) from the pancreatic lymph nodes (PLN) of 16 week-old mice was significantly lower in anti-IL-16 than control-treated mice (Fig. 4*A*). Thus, T-cells in anti-IL-16 treated mice are less activated at the time of destructive insulitis. Moreover, a lower percentage of $CD3^+$ T-cells and higher percentage of $B220^+$ B-cells was detected in islets of anti-IL-16 treated versus control-treated mice (supplementary Fig. 3*A*, available in an online appendix), decreasing the $CD3^+/B220^+$ ratio of islet-infiltrating cells from 2.2 to 1.1. Although $B220^+CD11c^{neg}$ immunosuppressive dendritic-like cells can prevent type 1 diabetes (18), we did not detect an increased frequency of this cellular subset in the pancreas of anti-IL-16 treated mice (supplementary Fig. 3*B*). In addition, a significant decrease occurred in the total number of islet-infiltrating $CD4^+$ ($P = 0.021$) and $CD8^+$ ($P = 0.001$) T-cells in anti-IL-16 treated mice without any significant change in the number of $B220^+$ B-cells ($P = 0.464$) (Fig. 4*B*), suggesting a preferential inhibitory effect on T-cells. Because CD4 can function as a chemotactic receptor for IL-16 on co-stimulated $CD8^+$ T-cells (19), neutralization of IL-16 may diminish the recruitment of activated $CD8^+$ T-cells to islets. Interestingly, a significantly altered chemokine profile was also observed in the PLN of anti-IL-16 treated mice (supplementary Table 1, available in an online appendix). Comparative analyses of changes in gene expression of 96 different cytokines and chemokines and their receptors performed using GEArray technology revealed that the expression of several transcripts, including CXCR1, CXCR3, CCR4, CCR5, CXCL9, CXCL10, CXCL12, CCL22, and macrophage migration inhibitory factor (MIF), was decreased >2-fold in anti-IL-16 versus control-treated mice. The activities of MIF, CCL22, CXCL10, CXCL12, ligands for CCR4 (CCL22), CCR5 (CCL5), and CXCR3 (CXCL10) have been associated with the development of type 1 diabetes (2,3,20–24). Only CCL4 and IL-10 mRNA expression was upregulated in the PLN of anti-IL-16 versus control-treated mice, which supports our later result that T-cell production of CCL4 is enhanced in the PLN of mice protected from type 1 diabetes. Note that, while a detectable increase in frequency of $CD4^+$ $IL\text{-}10^+$ T-cells in the PLN of anti-IL-16 treated mice was not detected, the mean fluorescent intensity (MFI) was increased, indicating that $CD4^+$ T-cells produce more IL-10 on a per cell basis. Moreover, in contrast to the spleen, diminished Th1-like responses were observed in the PLN of anti-IL-16 treated mice (supplementary Fig. 4, available in an online appendix). Thus, neutralization of IL-16 is associated with the decreased expression of several inflammatory gene transcripts in the PLN, where autoreactive T-cells may encounter antigen before migrating to islets and eliciting their destruction (25).

**Diabetogenic potential of spleen T-cells is reduced in anti-IL-16 treated mice.** Adoptive transfer studies were conducted to investigate whether a decrease in the diabe-

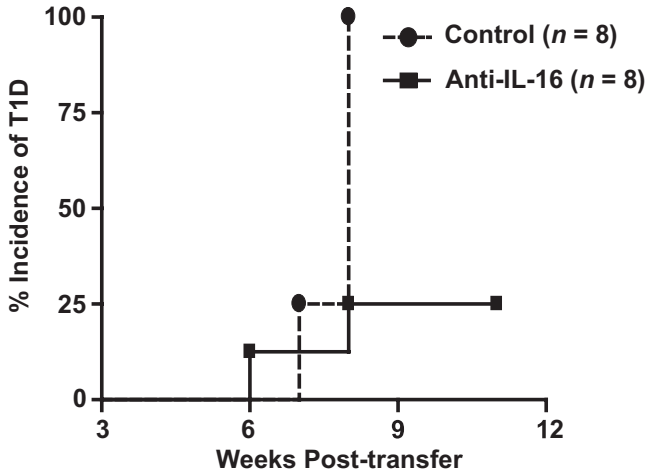


**FIG. 5. Diabetogenic potential of spleen T-cells is decreased in anti-IL-16 treated NOD mice. NOD mice were treated intraperitoneally with anti-IL-16 or isotype control antibody three times per week from 9 to 16 weeks of age, and their spleen T-cells were pooled at 16 weeks of age and transferred intraperitoneally to NOD.*Scid* recipients ($4 \times 10^6$ cells per mouse, eight mice per group). BGL were monitored until 11 weeks after transfer, and two consecutive BGLs ≥ 11.1 mmol/l were indicative of diabetes onset (*$P = 0.011$). Results are representative of three independent and reproducible experiments.**

togenic potential of spleen T-cells mediates anti-IL-16–induced protection against type 1 diabetes. Transfer of type 1 diabetes was significantly prevented ($P = 0.011$) in NOD.*Scid* recipients of spleen T-cells from anti-IL-16 treated NOD mice (16 weeks old) (Fig. 5). Whereas only 25% (2/8) of recipients of spleen T-cells from anti-IL-16 treated mice developed type 1 diabetes by 12 weeks after transfer, 100% (8/8) of NOD.*Scid* recipients of spleen T-cells from control-treated mice developed type 1 diabetes by 8 weeks after transfer. Thus, the diabetogenic potential of splenic T-cells is diminished appreciably in anti-IL-16–treated mice at 16 weeks of age. Because we did not detect a significant increase in the number of regulatory T-cells in the spleen of anti-IL-16 treated mice (our unpublished data), another mechanism(s) may control the diabetogenic potential of T-cells in anti-IL-16 treated mice.

**Anti-IL-16 therapy elicits Th1-like immune responses and susceptibility of T-cells to apoptosis.** Because Th2-type cytokine responses can prevent type 1 diabetes (26), we considered whether a heightened Th2-type immune response develops in anti-IL-16 treated mice. Unexpectedly, activated spleen T-cells from anti-IL-16 treated mice produced significantly more IL-2 ($P = 0.008$), IFN-γ ($P = 0.001$), and CCL4 ($P = 0.004$) than T-cells from control-treated mice (Fig. 6*A*). However, no changes in the level of IL-4 were detected (our unpublished data). Similarly, activated T-cells secreted elevated levels of CCL4, but not IL-4, in the PLN of anti-IL-16 treated mice (Fig. 6*B*). Administration of CCL4 directly prevents type 1 diabetes in NOD mice (27). Moreover, elevated levels of IFN-γ in the spleen diminish the autoimmune response in NOD mice by promoting the activation-induced cell death (AICD) of T-cells (28). Considering that IL-16 regulates susceptibility of $CD4^+$ T-cells to apoptosis (7), we determined whether anti-IL-16 enhances the apoptosis of T-cell receptor (TCR)-stimulated spleen cells. At 48 h poststimulation, significantly higher levels of apoptotic NOD spleen $CD4^+$ T-cells ($P = 0.008$) were detected in anti-IL-16 than in control-treated mice (Fig. 6*C*). Therefore, anti-IL-16 may protect from type 1 diabetes by enhancing $CD4^+$ T-cell susceptibility to apoptosis in response to TCR stimulation.

**Anti-IL-16 therapy inhibits the activation of autoreactive T-cells in the pancreas.** To further test the hypothesis that anti-IL-16 therapy regulates the recruitment and activation of islet-specific $CD4^+$ T-cells in the pancreas, we compared the levels of antigen-induced T-cell proliferation in the pancreas of anti-IL-16 and isotype control-treated mice. NOD mice were treated with anti-IL-16 or isotype control antibody between 3 and 15 weeks of age. At 14 weeks of age, both groups of NOD mice received $CD4^+CD25^{neg}$ T-cells from NOD.BDC2.5 $Thy1.1^+$ mice. At 7 days posttransfer, 73.5% of islet antigen specific $Thy1.1^+$BDC2.5 T-cells were dividing in the pancreas of isotype-treated mice compared to only 0.01% of the corresponding T-cells in anti-IL-16 treated

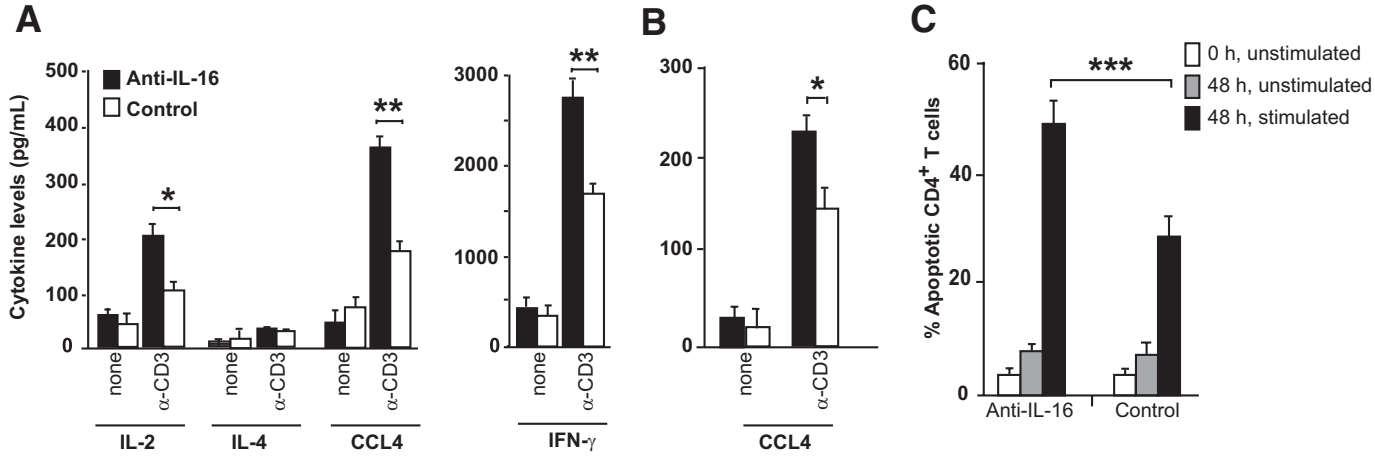


**FIG. 6. Anti-IL-16 treatment enhances Th1-type cytokine secretion and apoptosis of spleen T-cells. Spleen T-cells (*A*) or PLN-derived T-cells (*B*) ($10^6$/ml) purified from mice treated three times per week from 9 to 16 weeks of age with anti-IL-16 or control antibody were activated for 48 h in vitro with plate-bound anti-CD3ε. Cytokine and chemokine accumulation in supernatants from triplicate cultures were determined by ELISA. Mean values ± SD are shown, and similar results were obtained in two independent and reproducible experiments (*$P < 0.01$; **$P < 0.005$). *C*: Apoptosis of activated $CD4^+$ T-cells is augmented in anti-IL-16 treated mice. Spleen T-cells ($2 \times 10^6$ per ml) from anti-IL-16 or isotype Control-treated mice were stimulated with plate-bound anti-CD3ε, and at the indicated time points, the percentage of $CD4^+$ T-cells undergoing apoptosis was determined by transferase-mediated dUTP nick-end labeling staining. The results of triplicate cultures are expressed as the mean ± SEM (***$P = 0.005$). Results are representative of two independent and reproducible experiments.**

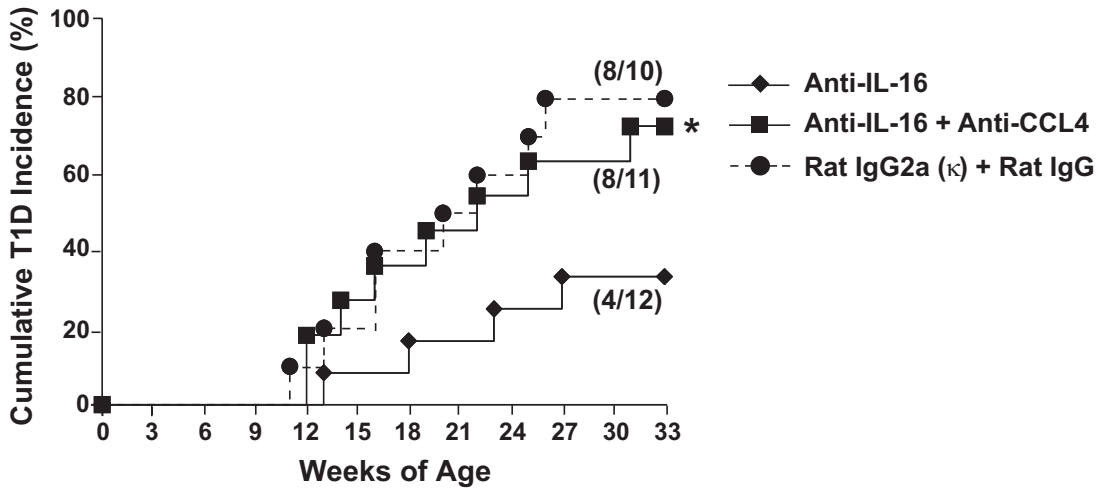


**FIG. 7. Anti-IL-16–induced protection from type 1 diabetes in NOD mice is CCL4-dependent. NOD mice were treated with 200 μg of the 14.1 anti-IL-16 mAb ($n$ = 12), 200 μg of anti-IL-16 plus 100 μg of anti-CCL4 ($n$ = 11), or 200 μg of isotype control IgG2a(κ) plus 100 μg control rat IgG ($n$ = 10) three times per week from 9 to 16 weeks of age. Mice were monitored for the development of type 1 diabetes until 33 weeks of age, and two consecutive BGLs of ≥11.1 mmol/l were indicative of onset of type 1 diabetes (*$P \leq 0.05$ relative to anti-IL-16 treated mice).**

recipients (supplementary Fig. 5, available in an online appendix). Minimal proliferation was observed in antigen-deficient inguinal lymph nodes (IGLN) in either isotype control or anti-IL-16 treated recipient mice. These results indicate that anti-IL-16 therapy inhibits the proliferation of islet antigen-specific T-cells in the pancreas.

**Neutralization of CCL4 abrogates anti-IL-16–induced protection against type 1 diabetes.** Because CCL4 protects from type 1 diabetes (27) and spleen- and PLN-T-cell production of CCL4 is elevated in anti-IL-16 mAb treated mice, we determined whether anti-IL-16–induced protection from type 1 diabetes occurs in a CCL4-dependent manner. The effect of coadministration of neutralizing anti-CCL4 and anti-IL-16 antibodies to female NOD mice from 9 to 16 weeks of age on the incidence of type 1 diabetes was evaluated. This combination treatment abrogated ($P$ = 0.05) the ability of anti-IL-16 to protect against type 1 diabetes (Fig. 7). Thus, anti-IL-16 therapy augments CCL4 production by T-cells, and anti-IL-16 mediated protection against type 1 diabetes is CCL4-dependent.

## DISCUSSION

In this study, we demonstrate that progression to the onset of type 1 diabetes in NOD mice is associated with an increased expression of IL-16 in pancreatic islets and identify IL-16 as a novel potential target for protection against the development of type 1 diabetes. Our studies localized mature IL-16 to B-cells and T-cells in islet lesions and detected IL-16 only during the development of invasive and destructive insulitis up to 15–16 weeks of age. Importantly, the secretion of IL-16 by several cell types in islet lesions can potentially augment the autoimmune response by recruiting additional $CD4^+$ cells, including various T-cell populations and dendritic cells, to sites of islet damage. Consistent with this idea, treatment of pre-diabetic NOD mice with a neutralizing anti-IL-16 mAb beginning at the outset of mature IL-16 expression in islets resulted in lower numbers of activated $CD4^+$ T-cells in the pancreas and provided optimum protection against destructive insulitis and type 1 diabetes. Consequently, we have demonstrated for the first time that neutralization of endogenous IL-16 activity may have therapeutic value for the treatment of type 1 diabetes because of its ability to arrest the transition from peri-insulitis to an invasive/destructive insulitis and onset of disease. This notion builds on the relationship between therapeutic efficacy and stage of disease treatment recommended for translational efficiency in clinical trials of type 1 diabetes (29). Furthermore, this relationship may apply for other successful modes of anti-IL-16 mediated protection, e.g., inflammation and disease in the experimental allergic encephalomyelitis (EAE) mouse model of multiple sclerosis and in a mouse model of inflammation-mediated ischemia–reperfusion injury of the kidney (30–32).

Although our study is the first to characterize the function of IL-16 during the development of type 1 diabetes, earlier reports have described how administration of a nondepleting anti-CD4 mAb also prevents type 1 diabetes (33,34). Similar to anti-IL-16 immunotherapy, this nondepleting anti-CD4 mAb prevents type 1 diabetes when administered after the development of invasive insulitis (33). The latter studies agree with our results considering that such an anti-CD4 mAb may block IL-16/CD4 interactions, and the efficacy of a nondepleting anti-CD4 mAb may be due in part to the inhibition of IL-16 function.

IL-16 can suppress $CD4^+$ T-cell activation and AICD (7), and such mechanisms may promote inflammation and/or autoimmunity by regulating T-cell homeostasis and limiting the clonal expansion of autoreactive T-cells (35,36). In NOD mice, the development of type 1 diabetes is influenced by defects in central and peripheral tolerance including TCR-stimulated $CD4^+$ T-cell resistance to apoptosis (37–41). Here, we showed that neutralization of IL-16 enhances TCR-induced susceptibility of spleen $CD4^+$ T-cells to apoptosis. Although the mechanism that regulates apoptosis of $CD4^+$ T-cells in anti-IL-16 treated mice is unknown, previous studies predict that CD95 may play a role (7). In addition, IFN-γ may also regulate T-cell apoptosis and homeostasis in anti-IL-16 treated mice given that we found an increased production of IFN-γ by spleen T-cells from anti-IL-16 versus control-treated mice. The latter finding is consistent with reports that IFN-γ induces the apoptosis of effector Th1 cells (42), triggers $CD4^+$ T-cell AICD (43,44), and protects from type 1 diabetes by

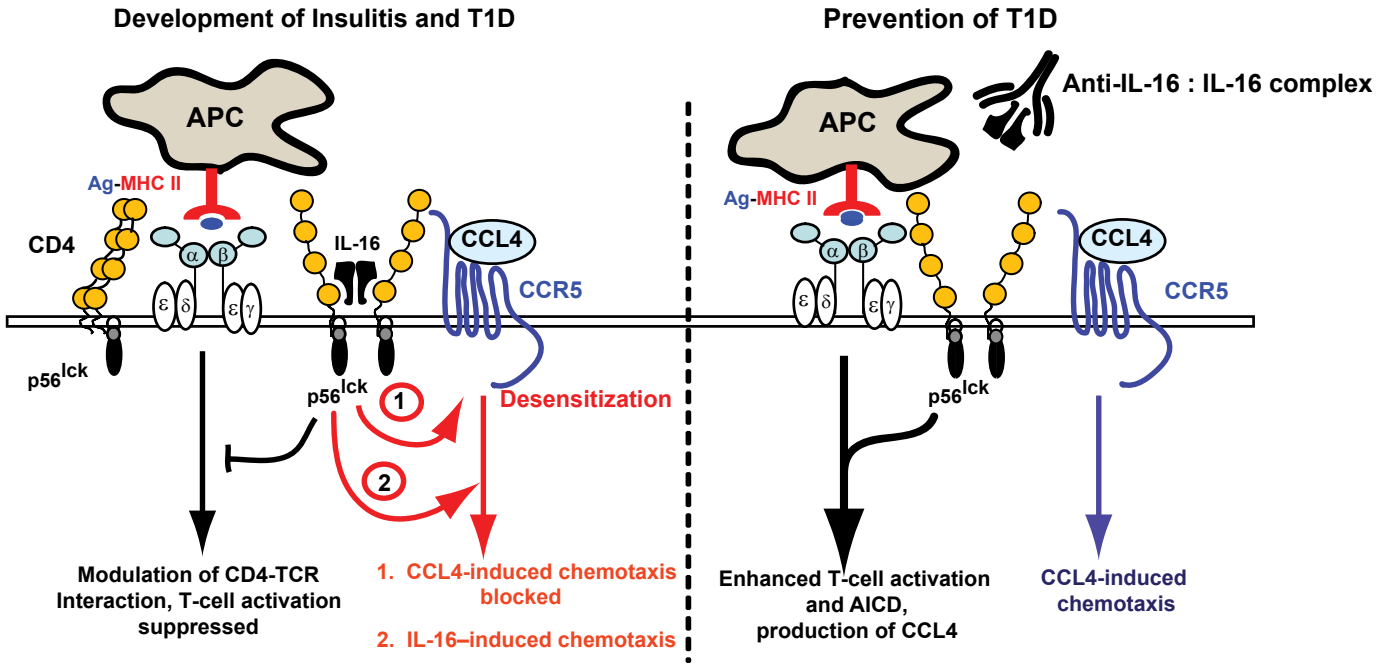


**FIG. 8. IL-16 and CCL4 may function as counterregulatory cytokines during the development of type 1 diabetes. Upon binding of IL-16 to CD4, interactions between CD4 and TCR are modulated as CD4 self-association is prevented and antigen-induced T-cell activation is suppressed. Concomitantly, a p56$^{lck}$ dependent signal desensitizes CCR5 and blocks the activity of CCL4 and other CCR5 ligands. A second nonp56$^{lck}$-dependent signal mediates IL-16–induced chemotaxis, which is enhanced by CCR5. Upon neutralization of IL-16, CD4 facilitates T-cell activation and CCL4 production is enhanced, resulting in the prevention of type 1 diabetes.**

enhancing the apoptosis of autoreactive spleen T-cells in NOD mice (28). Furthermore, we observed that spleen T-cells from anti-IL-16 treated mice were deficient in their ability to transfer type 1 diabetes to NOD.*Scid* mice. Thus, by enhancing susceptibility of CD4$^+$ effector T-cells to apoptosis together with neutralization of IL-16 chemotactic activity, anti-IL-16 therapy may reduce CD4$^+$ T-cell trafficking to islets by different mechanisms. Moreover, the reduced severity of islet infiltration by CD4$^+$ and CD8$^+$ T-cells in anti-IL-16 treated NOD mice is also accompanied by the decreased expression of several other inflammatory cytokines, chemokines, and their receptors in the pancreas (supplementary Table 1). Although neutralization of IL-16 protects from type 1 diabetes, we also noted that some anti-IL-16 treated mice developed type 1 diabetes at the same age as control-treated mice. It is likely that several other chemoattractant factors also target the immune system against islets in the absence of IL-16, which is consistent with the pleiotropic and redundant biology of chemoattractant cytokines.

A diminished inflammatory response against islets and prevention of type 1 diabetes seems to result in part from the decreased expression of several inflammatory mediators. Nonetheless, neutralization of IL-16 led to an increase in CCL4 and IL-10 expression in the PLN, which is relevant because CCL4 and IL-10 expression are associated with protection against type 1 diabetes. Of note, whereas activated T-cells were found to secrete elevated levels of CCL4 in the PLN of anti-IL-16 treated mice, CCL4 is also expressed by a variety of immune cells that could represent additional cellular sources of CCL4 in anti-IL-16 treated mice. Because we showed that neutralization of CCL4 reverses anti-IL-16 mediated protection against type 1 diabetes, our results agree with several reports that CCL4 protects against type 1 diabetes in mice and is not associated with disease onset in humans (22,27,45,46). Although an increase in the frequency of FoxP3$^+$ regulatory T-cells was not observed in anti-IL-16 treated mice, we cannot exclude the possibility that Tr1 regulatory T-cells and their secreted IL-10 product may be protective in these mice, as comparison of the IL-10 MFI indicated that CD4$^+$ T-cells do indeed produce greater amounts of IL-10 in the PLN of anti-IL-16 treated mice (supplementary Fig. 4).

Because CD4 and CCR5 can act synergistically to selectively recruit Th1 cells to sites of inflammation (4,47), CCL4 expression may be elevated in anti-IL-16 treated mice to compensate for deficient IL-16 activity. However, if IL-16 and CCL4 function cooperatively to promote islet inflammation, then neutralization of CCL4 would not be expected to abrogate anti-IL-16 mediated protection. Thus, our results support the notion that IL-16 may regulate or antagonize CCL4 function in the spleen and islets during the onset of type 1 diabetes. These findings are consistent with the observations that reciprocal cross-desensitization occurs between CD4 and CCR5 upon the binding of IL-16 and CCL4, respectively (15), and that CCL4 treatment can prevent type 1 diabetes (27). How CCL4 functions in anti-IL-16–induced protection from type 1 diabetes is currently not well understood, but this protection may result from the lack of IL-16–induced desensitization of CCR5 according to a model shown in Fig. 8 (4,15). This model proposes that CCL4 signaling through CCR5 mediates protection from type 1 diabetes, an idea supported by two recent reports that transient blockade of CCR5 with an anti-CCR5 mAb during 11–13 weeks of age or CCR5 deficiency significantly accelerates rather than prevents type 1 diabetes in NOD mice (48,49). Further experimentation is required to ascertain the functional role of CCL4 in anti-IL-16 mediated protection against type 1 diabetes.

## ACKNOWLEDGMENTS

This work was supported by grants from the Canadian Institutes of Health Research (MOP 64386), Juvenile Diabetes Research Foundation International, and Ontario Research and Development Challenge Fund (to T.L.D.). During these studies, T.L.D. was the Sheldon H. Weinstein Professor in Diabetes at the University of Western Ontario and C.M. was the recipient of the Canadian Diabetes Association Doctoral Student Award in honor of the late Helen E. Lewis. No potential conflicts of interest relevant to this article were reported.

C.M. researched data and wrote the manuscript. J.B., W.C., and N.H. researched data. G.A., Q.-S.M., and K.S. researched data and contributed to discussion. W.W.C. researched data and reviewed/edited the manuscript. T.L.D. wrote the manuscript and reviewed/edited the manuscript.

We thank all members of our laboratories for their continued support and advice during the preparation of this manuscript.

## REFERENCES

1. Bradley LM, Asensio VC, Schioetz LK, Harbertson J, Krahl T, Patstone G, Woolf N, Campbell IL, Sarvetnick N. Islet-specific Th1, but not Th2, cells secrete multiple chemokines and promote rapid induction of autoimmune diabetes. J Immunol 1999;162:2511–2520
2. Kim SH, Cleary MM, Fox HS, Chantry D, Sarvetnick N. CCR4-bearing T cells participate in autoimmune diabetes. J Clin Invest 2002;110:1675–1686
3. Lohmann T, Laue S, Nietzschmann U, Kapellen TM, Lehmann I, Schroeder S, Paschke R, Kiess W. Reduced expression of Th1-associated chemokine receptors on peripheral blood lymphocytes at diagnosis of type 1 diabetes. Diabetes 2002;51:2474–2480
4. Lynch EA, Heijens CA, Horst NF, Center DM, Cruikshank WW. Cutting edge: IL-16/CD4 preferentially induces Th1 cell migration: requirement of CCR5. J Immunol 2003;171:4965–4968
5. Rahangdale S, Morgan R, Heijens C, Ryan TC, Yamasaki H, Bentley E, Sullivan E, Center DM, Cruikshank WW. Chemokine receptor CXCR3 desensitization by IL-16/CD4 signaling is dependent on CCR5 and intact membrane cholesterol. J Immunol 2006;176:2337–2345
6. Kurschner C, Yuzaki M. Neuronal interleukin-16 (NIL-16): a dual function PDZ domain protein. J Neurosci 1999;19:7770–7780
7. Cruikshank WW, Kornfeld H, Center DM. Interleukin-16. J Leukoc Biol 2000;67:757–766
8. Chupp GL, Wright EA, Wu D, Vallen-Mashikian M, Cruikshank WW, Center DM, Kornfeld H, Berman JS. Tissue and T cell distribution of precursor and mature IL-16. J Immunol 1998;161:3114–3119
9. Baier M, Bannert N, Werner A, Lang K, Kurth R. Molecular cloning, sequence, expression, and processing of the interleukin 16 precursor. Proc Natl Acad Sci U S A 1997;94:5273–5277
10. Zhang Y, Center DM, Wu DM, Cruikshank WW, Yuan J, Andrews DW, Kornfeld H. Processing and activation of pro-interleukin-16 by caspase-3. J Biol Chem 1998;273:1144–1149
11. Wu DM, Zhang Y, Parada NA, Kornfeld H, Nicoll J, Center DM, Cruikshank WW. Processing and release of IL-16 from CD4+ but not CD8+ T cells is activation dependent. J Immunol 1999;162:1287–1293
12. Cruikshank W, Center DM. Modulation of lymphocyte migration by human lymphokines. II. Purification of a lymphotactic factor (LCF). J Immunol 1982;128:2569–2574
13. Center DM, Cruikshank W. Modulation of lymphocyte migration by human lymphokines. I. Identification and characterization of chemoattractant activity for lymphocytes from mitogen-stimulated mononuclear cells. J Immunol 1982;128:2563–2568
14. Cruikshank WW, Lim K, Theodore AC, Cook J, Fine G, Weller PF, Center DM. IL-16 inhibition of CD3-dependent lymphocyte activation and proliferation. J Immunol 1996;157:5240–5248
15. Mashikian MV, Ryan TC, Seman A, Brazer W, Center DM, Cruikshank WW. Reciprocal desensitization of CCR5 and CD4 is mediated by IL-16 and macrophage-inflammatory protein-1 beta, respectively. J Immunol 1999; 163:3123–3130
16. Van Drenth C, Jenkins A, Ledwich L, Ryan TC, Mashikian MV, Brazer W, Center DM, Cruikshank WW. Desensitization of CXC chemokine receptor 4, mediated by IL-16/CD4, is independent of p56lck enzymatic activity. J Immunol 2000;165:6356–6363
17. Seegert D, Rosenstiel P, Pfahler H, Pfefferkorn P, Nikolaus S, Schreiber S. Increased expression of IL-16 in inflammatory bowel disease. Gut 2001;48: 326–332
18. Liang X, Ma L, Thai NL, Fung JJ, Qian S, Lu L. The role of liver-derived regulatory dendritic cells in prevention of type 1 diabetes. Immunology 2007;120:251–260
19. Kitchen SG, LaForge S, Patel VP, Kitchen CM, Miceli MC, Zack JA. Activation of CD8 T cells induces expression of CD4, which functions as a chemotactic receptor. Blood 2002;99:207–212
20. Bojunga J, Kusterer K, Bacher M, Kurek R, Usadel KH, Renneberg H. Macrophage migration inhibitory factor and development of type-1 diabetes in non-obese diabetic mice. Cytokine 2003;21:179–186
21. Matin K, Salam MA, Akhter J, Hanada N, Senpuku H. Role of stromal-cell derived factor-1 in the development of autoimmune diseases in non-obese diabetic mice. Immunology 2002;107:222–232
22. Cameron MJ, Arreaza GA, Grattan M, Meagher C, Sharif S, Burdick MD, Strieter RM, Cook DN, Delovitch TL. Differential expression of CC chemokines and the CCR5 receptor in the pancreas is associated with progression to type I diabetes. J Immunol 2000;165:1102–1110
23. Christen U, McGavern DB, Luster AD, von Herrath MG, Oldstone MB. Among CXCR3 chemokines, IFN-gamma-inducible protein of 10 kDa (CXC chemokine ligand (CXCL) 10) but not monokine induced by IFN-gamma (CXCL9) imprints a pattern for the subsequent development of autoimmune disease. J Immunol 2003;171:6838–6845
24. Frigerio S, Junt T, Lu B, Gerard C, Zumsteg U, Holländer GA, Piali L. Beta cells are responsible for CXCR3-mediated T-cell infiltration in insulitis. Nat Med 2002;8:1414–1420
25. Höglund P, Mintern J, Waltzinger C, Heath W, Benoist C, Mathis D. Initiation of autoimmune diabetes by developmentally regulated presentation of islet cell antigens in the pancreatic lymph nodes. J Exp Med 1999;189:331–339
26. Cameron MJ, Arreaza GA, Zucker P, Chensue SW, Strieter RM, Chakrabarti S, Delovitch TL. IL-4 prevents insulitis and insulin-dependent diabetes mellitus in nonobese diabetic mice by potentiation of regulatory T helper-2 cell function. J Immunol 1997;159:4686–4692
27. Meagher C, Arreaza G, Peters A, Strathdee CA, Gilbert PA, Mi QS, Santamaria P, Dekaban GA, Delovitch TL. CCL4 protects from type 1 diabetes by altering islet beta-cell-targeted inflammatory responses. Diabetes 2007;56:809–817
28. Sobel DO, Han J, Williams J, Yoon JW, Jun HS, Ahvazi B. Gamma interferon paradoxically inhibits the development of diabetes in the NOD mouse. J Autoimmun 2002;19:129–137
29. Shoda LK, Young DL, Ramanujan S, Whiting CC, Atkinson MA, Bluestone JA, Eisenbarth GS, Mathis D, Rossini AA, Campbell SE, Kahn R, Kreuwel HT. A comprehensive review of interventions in the NOD mouse and implications for translation. Immunity 2005;23:115–126
30. Skundric DS, Cai J, Cruikshank WW, Gveric D. Production of IL-16 correlates with CD4+ Th1 inflammation and phosphorylation of axonal cytoskeleton in multiple sclerosis lesions. J Neuroinflammation 2006;3:13
31. Skundric DS, Dai R, Zakarian VL, Bessert D, Skoff RP, Cruikshank WW, Kurjakovic Z. Anti-IL-16 therapy reduces CD4+ T-cell infiltration and improves paralysis and histopathology of relapsing EAE. J Neurosci Res 2005;79:680–693
32. Wang S, Diao H, Guan Q, Cruikshank WW, Delovitch TL, Jevnikar AM, Du C. Decreased renal ischemia-reperfusion injury by IL-16 inactivation. Kidney Int 2008;73:318–326
33. Hutchings P, O'Reilly L, Parish NM, Waldmann H, Cooke A. The use of a non-depleting anti-CD4 monoclonal antibody to re-establish tolerance to beta cells in NOD mice. Eur J Immunol 1992;22:1913–1918
34. Liu E, Moriyama H, Paronen J, Abiru N, Miao D, Yu L, Taylor RM, Eisenbarth GS. Nondepleting anti-CD4 monoclonal antibody prevents diabetes and blocks induction of insulin autoantibodies following insulin peptide B:9–23 immunization in the NOD mouse. J Autoimmun 2003;21: 213–219
35. Van Parijs L, Abbas AK. Homeostasis and self-tolerance in the immune system: turning lymphocytes off. Science 1998;280:243–248
36. Chervonsky AV. Apoptotic and effector pathways in autoimmunity. Curr Opin Immunol 1999;11:684–688
37. Rapoport MJ, Jaramillo A, Zipris D, Lazarus AH, Serreze DV, Leiter EH, Cyopick P, Danska JS, Delovitch TL. Interleukin 4 reverses T cell proliferative unresponsiveness and prevents the onset of diabetes in nonobese diabetic mice. J Exp Med 1993;178:87–99
38. Decallonne B, van Etten E, Giulietti A, Casteels K, Overbergh L, Bouillon R, Mathieu C. Defect in activation-induced cell death in non-obese diabetic (NOD) T lymphocytes. J Autoimmun 2003;20:219–226
39. Salojin KV, Zhang J, Madrenas J, Delovitch TL. T-cell anergy and altered T-cell receptor signaling: effects on autoimmune disease. Immunol Today 1998;19:468–473

40. Arreaza G, Salojin K, Yang W, Zhang J, Gill B, Mi QS, Gao JX, Meagher C, Cameron M, Delovitch TL. Deficient activation and resistance to activation-induced apoptosis of CD8+ T cells is associated with defective peripheral tolerance in nonobese diabetic mice. Clin Immunol 2003;107:103–115
41. Noorchashm H, Moore DJ, Noto LE, Noorchashm N, Reed AJ, Reed AL, Song HK, Mozaffari R, Jevnikar AM, Barker CF, Naji A. Impaired CD4 T cell activation due to reliance upon B cell-mediated costimulation in nonobese diabetic (NOD) mice. J Immunol 2000;165:4685–4696
42. Liu Y, Janeway CA Jr. Interferon gamma plays a critical role in induced cell death of effector T cell: a possible third mechanism of self-tolerance. J Exp Med 1990;172:1735–1739
43. Dalton DK, Haynes L, Chu CQ, Swain SL, Wittmer S. Interferon gamma eliminates responding CD4 T cells during mycobacterial infection by inducing apoptosis of activated CD4 T cells. J Exp Med 2000;192:117–122
44. Refaeli Y, Van Parijs L, Alexander SI, Abbas AK. Interferon gamma is required for activation-induced death of T lymphocytes. J Exp Med 2002;196:999–1005
45. Bergerot I, Arreaza GA, Cameron MJ, Burdick MD, Strieter RM, Chensue SW, Chakrabarti S, Delovitch TL. Insulin B-chain reactive CD4+ regulatory T-cells induced by oral insulin treatment protect from type 1 diabetes by blocking the cytokine secretion and pancreatic infiltration of diabetogenic effector T-cells. Diabetes 1999;48:1720–1729
46. Pfleger C, Kaas A, Hansen L, Alizadeh B, Hougaard P, Holl R, Kolb H, Roep BO, Mortensen HB, Schloot NC, Hvidøre Study Group On Childhood Diabetes. Relation of circulating concentrations of chemokine receptor CCR5 ligands to C-peptide, proinsulin and HbA1c and disease progression in type 1 diabetes. Clin Immunol 2008;128:57–65
47. Staudinger R, Phogat SK, Xiao X, Wang X, Dimitrov DS, Zolla-Pazner S. Evidence for CD4-enchanced signaling through the chemokine receptor CCR5. J Biol Chem 2003;278:10389–10392
48. Seifarth C, Mack M, Steinlicht S, Hahn EG, Lohmann T. Transient chemokine receptor blockade does not prevent, but may accelerate type 1 diabetes in prediabetic NOD mice. Horm Metab Res 2006;38:167–171
49. Solomon M, Balasa B, Sarvetnick N. CCR2 and CCR5 chemokine receptors differentially influence the development of autoimmune diabetes in the NOD mouse. Autoimmunity 2010;43:156–163