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Differential Expression of CC Chemokines and the CCR5 Receptor in the Pancreas Is Associated with Progression to Type I Diabetes

Mark J. Cameron,*‡ Guillermo A. Arreaza,‡ Marsha Grattan,* Craig Meagher,*† Shayan Sharif,* Marie D. Burdick,‡ Robert M. Strieter,§ Donald N. Cook,§* and Terry L. Delovitch*‡

We investigated the biological role of CC chemokines in the Th1-mediated pathogenesis of spontaneous type I diabetes in nonobese diabetic (NOD) mice. Whereas an elevated ratio of macrophage inflammatory protein-1α (MIP-1α):MIP-1β in the pancreas correlated with destructive insulitis and progression to diabetes in NOD mice, a decreased intrapancreatic MIP-1α:MIP-1β ratio was observed in nonobese diabetes-resistant (NOR) mice. IL-4 treatment, which prevents diabetes in NOD mice by polarizing intraislet Th2 responses, decreased CCR5 expression in islets and potentiated a high ratio of MIP-1α and monocyte chemotactic protein-1 (MCP-1):MIP-1α in the pancreas. Furthermore, NOD.MIP-1α−/− mice exhibited reduced destructive insulitis and were protected from diabetes. Neutralization of MIP-1α with specific Abs following transfer of diabetogenic T cells delayed the onset of diabetes in NOD.Scid recipients. These studies illustrate that the temporal expression of certain CC chemokines, particularly MIP-1α, and the CCR5 chemokine receptor in the pancreas is associated with the development of insulitis and spontaneous type I diabetes.

Chemokines mediate innate and adaptive immune responses by their ability to recruit, activate, and costimulate T cells and monocytes (1, 2). Depending on the juxtaposition of the first two cysteine residues in the amino acid sequence, chemokines are divided into four classes: the CXC, CC, C, and CX3C families. Macrophage inflammatory protein-1α (MIP-1α), MIP-1β, and monocyte chemotactic protein-1 (MCP-1) are representative of the CC family of chemokines that characterizes Th2 cells (8–11). In addition, chemokine receptor expression can be modulated by cytokines known to influence T cell polarization, e.g., IFN-γ, IL-4, and TGF-β (12, 13).

Nonobese diabetic (NOD) mice spontaneously develop a form of type I diabetes that shares many features of the human disease (14). Mononuclear cell infiltration of pancreatic islets and the progressive Th1 cell-mediated destruction of insulin-producing β cells herald the onset of autoimmune type I diabetes. Previously, we found that the genetic control of NOD T cell proliferative hyporesponsiveness to engagement of the TCR is linked to a central region on chromosome 11 that includes the CC chemokine gene family and the Idd4 diabetes susceptibility locus (15). We also found that IL-4 treatment and CD28 costimulation prevent Th1-mediated destructive insulitis and type I diabetes in NOD mice by potentiation of regulatory Th2 cell function (16–18). IL-4 (19) and CD28 (20) can each modulate CC chemokine expression. Given the role of CC chemokines in inflammation and autoimmunity, we analyzed the expression of certain of these chemokines and their receptors in the pancreas of NOD mice at the preinsulitis (0–5 wk), nondestructive insulitis (5–12 wk), and destructive insulitis (>12 wk) inflammatory stages of onset of diabetes. In particular, we determined whether the intrapancreatic expression of selected CC chemokines and CCR receptors is associated with the progression to diabetes onset. Our results demonstrate that temporal expression of MIP-1α and its CCR5 receptor contributes to the development of diabetes.
of destructive insulitis and spontaneous type I diabetes in NOD mice.

Materials and Methods

Mice

NOD/Scid, nonobese diabetes-resistant (NOR), and severe combined immunodeficient NOD (NOD.Scid) female mice were bred in a specific pathogen-free barrier facility at The John P. Robarts Research Institute (London, Canada). Islet infiltration begins at 4–6 wk of age in our colony of female NOD mice, and progression to destructive insulitis and overt diabetes occurs by 4–6 mo of age. The incidence of diabetes in female NOD mice in our colony is 40–50% at 15 wk of age and 80–90% by 25 wk. C57BL/6 and 129/J mice were obtained from The Jackson Laboratory (Bar Harbor, ME).

IL-4 treatment

Fifty nanograms (500 U) of recombinant murine IL-4 (Immunex, Seattle, WA) in 0.2 ml of vehicle (PBS plus 1% serum from ≤6-wk-old prediabetic female NOD mice) was administered i.p. three times weekly to female NOD mice from 2 wk (preinsulitis) to 10 wk of age as previously described (18). Control mice received only vehicle.

Assay of chemokine protein content in tissues

Pancreata and spleens from 1) age-matched nondiabetic NOD, NOR, and NOD.Scid mice; 2) nondiabetic and diabetic female NOD littermates; 3) nondiabetic IL-4–treated and control nondiabetic vehicle-treated NOD females; and 4) age-matched nondiabetic and diabetic female NOD.MIP-1α−/− and NOD.MIP-1α+/− mice were snap-frozen in liquid N2. Immediately before analysis, tissues were homogenized and sonicated in an antiprotease buffer (Roche, Laval, Canada) as previously described (21–24). Homogenates were centrifuged to remove debris and were then passed through 1.2-μm pore size filters (Gelman Sciences, Ann Arbor, MI). Chemokine concentrations were determined by an ELISA using polyclonal 145-2C11 anti-CD3 antibody (American Type Culture Collection, Rockville, MD) and the plate-bound 145-2C11 anti-CD3 antibody (American Type Culture Collection, Rockville, MD) as previously described. Standard curves were linear in the range of 20–2000 pg/ml. ELISA results were normalized relative to the total protein (Bradford dye-binding protein assay, Bio-Rad, Mississauga, Canada) derived from each tissue.

Assay of chemokine receptor expression

Splenic T cells were purified on T cell purification columns (R&D Systems) to a purity of ≥98%, as assayed by FACS analysis of CD3 cell surface expression. Islets were isolated as previously described (18). Total RNA was purified from tissues by the guanidinium isothiocyanate/silica gel-based membrane RNeasy method (Qiagen, Valencia, CA) using the manufacturer’s recommended procedure except that 0.14 M 2-ME was added to the antiprotease buffer (Roche, Laval, Canada). RNA was purified from tissues by the guanidinium isothiocyanate/silica gel-based membrane RNeasy method (Qiagen, Valencia, CA) using the manufacturer’s recommended procedure except that 0.14 M 2-ME was added to the antiprotease buffer (Roche, Laval, Canada) as previously described (21-24). Chemokines were amplified, and the products were separated on an agarose gel. Ethidium bromide-stained product intensities of the MIMIC and cDNA targets were quantified by Gel Doc 1000 video gel documentation (Bio-Rad, Hercules, CA). The amount of target cDNA was determined by comparing the amount of MIMIC required to produce equimolar quantities of CCR5 gene or CCR2 product. The positions of migration of the MIMIC and cDNA targets were determined relative to DNA m.w. standards of 100–1500 bp (Life Technologies).

Adaptive T cell transfer

Female NOD.Scid mice (n = 6/group) at 8–10 wk of age were injected i.p. with splenic T cells (5 × 106) from newly diabetic female NOD mice. Recipient mice received injections (100 μg/injection) of either a neutralizing anti-mouse anti-MIP-1α or anti-MIP-1β polyclonal Ab (R&D Systems) or control normal goat IgG (Cedarlane) three times a week for 3 wk. Beginning at 2 wk post-transfer, mice were monitored twice weekly for the presence of hyperglycemia for up to 10 wk. A blood glucose level reading of ≥11.1 mmol/L glucose on two consecutive occasions was indicative of the onset of diabetes.

Generation of NOD.MIP-1α−/− mice

C57BL/6.MIP-1α−/− mice, previously shown to be resistant to virus-induced autoimmune inflammation, were generated in 129 embryonic stem cells by gene-targeted disruption (25). Mice were backcrossed six generations onto the NOD background (>98% NOD-like; Idd1−7, Idd10, Idd12, Idd14, and Idd15 positive) and intercrossed to generate mice with the NOD.MIP-1α−/− and NOD.MIP-1α+/− genotypes. Genotyping was performed using PCR primers specific for the MIP-1α−/− genotype (Neo forward primer (5′-TAAAGGCGATGCCTCAGACT-3′) and MIP-1α reverse primer (5′-GAATTGCGAGAAGTCTCACTCCA-3′)) or the MIP-1α−/− genotype (MIP-1α 542 forward primer (5′-CCTCTCATGTTGGAAGG-3′) and MIP-1α reverse primer (5′-GAAGTGGGAACTGACTTACTCCA-3′)). Specific primers were also generated for the PCR genotyping of the D11 Ndsl (43.7 cm), D11 Mit38 (49.0 cm), and D11 Mit325 (49.0 cm) polymorphic microsatellite markers in the Idd4 diabetes susceptibility locus of 129/J, C57BL/6, and NOD mice. The chromosomal positions of these markers (indicated in parentheses) were determined according to the most current map of mouse chromosome 11 (Mouse Genome Database, Mouse Genome Informatics, The Jackson Laboratory (http://www.informatics.jax.org)). These analyses narrowed the region of interest to a chromosomal distance of about 5–6 cm.

To address the possibility that the diabetes phenotype observed in NOD.MIP-1α−/− mice may be due to a 129I-derived (disease-resistant) linked gene(s), we generated a control mouse strain, designated NOD.129/J, by backcrossing 129/J to NOD mice for six generations. Mice that expressed 129/J-derived alleles at the CC chemokine locus on mouse chromosome 11 were selected at each generation. Microsatellite markers used to genotype this region of chromosome 11 were D11 Mit325, D11 Mit98, and D11 Mit258.

Histopathology analysis

Pancreata were removed, fixed with 10% buffered formalin, embedded in paraffin, and sectioned at 5-μm intervals. The incidence and severity of insulitis were examined by hematoxylin and eosin staining as well as in-situ immunostaining. A minimum of 30 islets from each mouse were observed, and the degree of mononuclear cell infiltration was scored independently and blindly by two observers using the following ranking: 0, no infiltration of the islet architecture; 2, moderate insulitis, but no infiltration of the islet architecture; 2, moderate insulitis (mononuclear cell infiltration <50% of the islet architecture); and 3, severe insulitis (>50% of the islet tissue infiltrated by lymphocytes accompanied by a reduction in insulin staining). The immunohistochemical identification of insulin was performed using a porcine anti-insulin Ab and avidin-biotin peroxidase detection system (Dako, Carpinteria, CA).

Statistical analysis

Results were compared using Student’s t test for unpaired samples. p < 0.05 was chosen as the level of significance.

Results

Early pancreas inflammation correlates with a high MIP-1α: MIP-1β ratio in NOD mice

To identify candidate CC chemokines that are associated with the establishment and/or progression of insulitis, the intrapancreatic levels of MIP-1α, MIP-1β, and MCP-1 in prediabetic female
NOD, NOR, and NOD.Scid mice (lack functional T and B cells, insulinis- and diabetes-free) were quantified in whole tissue homogenates by ELISA. NOD and NOR mice are MHC congenic and share 88% of their genomes, but islet inflammation does not progress beyond a nondestructive peri-insulitis in NOR mice (26).

Age-dependent analyses showed that relative to the pancreas of NOR and NOD.Scid mice, high levels of MIP-1α (600 pg/mg tissue) are detectable in pancreata of female NOD mice at 5 wk of age during the onset of insulitis (Fig. 1A). At 10 wk of age, the expression of MIP-1α in NOR pancreata increased about 3-fold to the level found in NOD pancreata. In contrast, a 3-fold higher intrapancreatic level of MIP-1β was observed in NOR (1200 pg/mg tissue) than in NOD (400 pg/mg tissue) mice at 5 wk of age, while a 4-fold higher level (2300 vs 575 pg/mg tissue) was observed in 10-wk-old mice (Fig. 1B). Equivalent levels of MCP-1 were found in the pancreata of newborn to 10-wk-old NOR, NOD, and NOD.Scid mice. MCP-1 content increased starting at 15 wk of age to about 450 pg/mg tissue in 20-wk-old NOR mice and 750 pg/mg tissue in 20-wk-old NOD and NOD.Scid mice, respectively (Fig. 1D). Interestingly, C57BL/6j mice between 5 and 30 wk of age do not express MIP-1α, MIP-1β, or MCP-1 in the pancreas (our unpublished observations). This result demonstrates the importance of the NOD genetic background in the control of the patterns of CC chemokine expression observed in the pancreas of NOD, NOR, and NOD.Scid mice. Thus, during the initial stages of islet mononuclear cell infiltration (≤10 wk of age), the progression to destructive insulitis in NOD mice vs a benign peri-insulitis in NOR mice is associated with a higher intrapancreatic MIP-1α: MIP-1β ratio in NOD mice despite the presence of similar pancreatic levels of MCP-1 in NOD and NOR mice at this time (Fig. 1, C–E).

**IL-4 therapy modulates intrapancreatic chemokine content in NOD mice**

Since we have shown that IL-4 treatment protects female NOD mice from diabetes by stabilizing a Th2-like cytokine environment in the pancreas (18), we determined whether this IL-4-mediated protection is accompanied by changes in the levels of intrapancreatic expression of chemokines. At 5 wk of age, MIP-1α was reduced about 3-fold in IL-4-treated compared with control NOD mice (Fig. 3A), and MIP-1β was increased about 6-fold in 10-wk-old IL-4-treated female NOD mice (Fig. 3B). With the exception that a 2-fold increase in the intrapancreatic content of MCP-1 was detected in 10- and 20-wk-old NOD mice treated with IL-4 (Fig. 3D), this chemokine profile was comparable to that found in NOR mice (Fig. 1, A and B). At 10 wk of age, MIP-1α content was reduced about 2-fold in the spleen of IL-4-treated female NOD mice relative to that in control mice, while MIP-1β and MCP-1 levels were similar in IL-4-treated and control mice (data not shown). Thus, a reduced ratio of MIP-1α:MIP-1β plus MCP-1 and a late increase in MCP-1 expression in the pancreas following IL-4 treatment is associated with protection from diabetes (Fig. 3, C–E).
per milligram) total tissue protein were quantitated by ELISA. Values are expressed as the mean (picograms per milligram) total tissue protein ± SEM or as MIP-1α:MIP-1β and MIP-1α:MCP-1 ratios ± SEM. Data from one of two representative experiments are shown. **, p ≤ 0.01.

Islet-infiltrated T cells from IL-4-treated mice are Th2-like and produce high levels of MIP-1β

Islet-infiltrated T cells from 10-wk-old IL-4-treated NOD mice secreted higher levels of IL-4 (≥5-fold increase) and MIP-1β (~50-fold) compared with islet-infiltrated T cells from age-matched control mice (Fig. 4). IFN-γ secretion by islet-infiltrated T cells was detectable (5000 pg/ml) only in 10-wk-old control mice. Less MIP-1α was secreted by islet-infiltrated T cells of IL-4-treated mice than in control mice; however, the observed differences were generally less significant (20–25% decrease). The levels of secretion of MCP-1 (Fig. 4) as well as the magnitude of proliferative responses by islet-infiltrated T cells from IL-4-treated and control NOD mice did not differ significantly (our unpublished observations). Interestingly, basal T cell proliferative responses in IL-4-treated and control NOD mice were generally higher than those of splenic T cells in parallel cultures (data not shown). The latter result probably reflects the increased activity of T cells isolated from a site of inflammation and may explain the high basal chemokine production observed in Fig. 4. Thus, the results shown in Fig. 4 are compatible with those obtained for the CC chemokine levels (Fig. 3) and cytokine content (18) in the pancreas of IL-4-treated NOD mice of similar age and support the idea that a higher IL-4 plus MIP-1β:IFN-γ plus MIP-1α ratio is associated with protection from diabetes.

IL-4 treatment reduces CCR5 mRNA expression in NOD splenic T cells and islets

In addition to cytokine and chemokine expression, the abilities of different T cell subsets to migrate to sites of inflammation depend on their expression of certain chemokine receptors (1, 12). To further investigate how IL-4 treatment affects lymphocyte recruitment to the pancreas and prevents diabetes, we compared the expression of CC chemokine receptor mRNA in splenic T cells and islets from IL-4-treated and control female NOD mice by semi-quantitative RT-PCR. While no reproducible differences in the expression of CCR1, CCR2, CCR3, CCR4, CXCR3, and CXCR4 were observed using this method, a consistent reduction in CCR5 mRNA expression was detected in splenic T cells and islets of IL-4-treated mice (our unpublished observations). We therefore used a MIMIC RT-PCR technique to determine more quantitatively the difference in CCR5 mRNA levels noted between splenic T cells and islets of IL-4-treated and control NOD mice. The level of CCR5 mRNA was reduced about 4-fold in islets from 10-wk-old IL-4-treated NOD mice compared with that expressed by islets from control mice (Fig. 5A, lane 2 vs lane 11). No significant changes in mRNA expression of the control CCR2 were detected in islets from IL-4-treated mice (Fig. 5B). Similarly, a 4-fold reduction in the expression of CCR5 mRNA (Fig. 5C, lane 3 vs lane 12), but not CCR2 mRNA (Fig. 5D), was detected in splenic T cells from 10-wk-old IL-4-treated vs control NOD mice. Thus, the mRNA expression of CCR5, a CC chemokine receptor associated with Th1-type immune responses (9–11), is selectively down-regulated in splenic T cells and islets of NOD mice treated with IL-4.

MIP-1α plays a role in the pathogenesis of type 1 diabetes

We adopted two approaches to investigate whether a cause-and-effect relationship exists between the level of expression of MIP-1α in pancreatic islets and the development of type I diabetes. First, NOD.Scid recipients of splenic T cells (5 × 10⁶) from diabetic NOD mice received injections (100 μg/injection) of either a neutralizing polyclonal goat anti-mouse anti-MIP-1α Ab or control normal goat IgG three times weekly for 3 wk. At 4 wk after the transfer of diabetogenic T cells, the incidence of diabetes was 100% in control IgG-treated mice (Fig. 6). Whereas the incidence of diabetes was significantly reduced from 100 to 50% in anti-MIP-1α-treated mice at 8 wk post-cell transfer, anti-MIP-1β treatment provided only minimal protection, yielding an incidence of 85% diabetes at this time.
peri-insulitis was evident in only 30% of islets from NOD.MIP-1a/litter of pancreas from NOD.MIP-1 mice. Histological sections of pancreas from NOD.MIP-1a/litter were included as negative controls. Each reaction contained equivalent amounts of RNA, as confirmed by amplification of the housekeeping gene GAPDH. M, position of migration of 100- to 1500-bp DNA m.w. standards. Results were confirmed within each experiment, and data from one of three representative experiments are shown.

Second, we monitored islet inflammation and the spontaneous incidence of diabetes in NOD.MIP-1a/litter mice. Histological sections of pancreas from NOD.MIP-1a/litter and NOD.MIP-1a/litter mice were scored for insulitis at 10 wk of age. While 60% of the islets displayed either normal histology or peri-insulitis in the pancreas of 10-wk-old NOD.MIP-1a/litter mice, a normal histology or peri-insulitis was evident in only 30% of islets from NOD.MIP-1a/litter mice (Fig. 7A). Conversely, the percentage (40%) of islets displaying moderate or severe insulitis in 10-wk-old NOD.MIP-1a/litter mice was also lower than that (70%) observed in NOD.MIP-1a/litter mice. To test the statistical significance of these data, the severity of islet inflammation was also scored as nondestructive (combined scores of 0 and 1) and destructive (combined scores of 2 and 3) infiltration (Fig. 7B). Note that 10-wk-old NOD.MIP-1a/litter mice exhibit a significantly higher proportion of islets of a nondestructive phenotype (p < 0.01) and a significantly lower proportion of islets of a destructive phenotype (p < 0.01) relative to NOD.MIP-1a/litter mice. Thus, deficient MIP-1a expression in NOD mice reduces the amount of destructive insulitis in the pancreas.

The incidence of diabetes monitored as previously described (18) was significantly reduced and delayed in NOD.MIP-1a/litter female mice compared with NOD.MIP-1a/litter mice (Fig. 7C). We determined that NOD.MIP-1a/litter mice express the Idd1, Idd2, Idd3, Idd4, Idd5, Idd6, Idd7, Idd10, Idd12, and Idd14 diabetes susceptibility loci. Despite the observed lower incidence of 60% diabetes in NOD.MIP-1a/litter mice compared with the 80–90% incidence noted in wild-type NOD female mice, we found that the incidence of diabetes approached 90% in three of the seven NOD.MIP-1a/litter litters. The overall lower incidence of diabetes in NOD.MIP-1a/litter mice may result from the incomplete fixation of other Idd loci in these mice. Clinical determinations of these mice following the onset of overt diabetes, including blood glucose measurements, physical assessments, and response to insulin therapy, have not revealed any differences in the severity of disease between NOD.MIP-1a/litter and NOD.MIP-1a/litter mice.

The Idd4 locus located on mouse chromosome 11 encompasses the CC chemokine gene family. This raises the possibility that 129- or C57BL/6-derived Idd4 genes cosegregated with the mutated MIP-1a gene during the backcrossing of this gene onto the NOD.
genetic background. To examine whether such 129/J- or C57BL/6-derived cosegregating genes influence the incidence of diabetes in our NOD.MIP-1α+/− mice or NOD.MIP-1α−/− mice, we analyzed the genotype in chromosome 11 regions centromeric and telomeric to the CC chemokine locus. We found that there is probably minimal influence of these cosegregating genes on the incidence of diabetes, since regions flanking this chemokine locus were genotyped as being NOD in origin. Thus, the Idd4 locus in NOD.MIP-1α−/− mice was derived from the NOD strain and could not account for the phenotype of NOD.MIP-1α−/− mice. Since Idd4 may mask the effects of cosegregating 129/J genes on chromosome 11, we reasoned that the NOD.MIP-1α+/− strain is an appropriate genetic control for NOD.MIP-1α−/− mice. A similar approach was previously used to rule out possible effects of the Idd6 locus cosegregating with a mutated TNFR1 gene on mouse chromosome 6 in TNFR1-deficient mice (27). The possibility that undefined resistant Idd alleles were linked to a mutated CIITA gene on mouse chromosome 16 in CIITA-deficient mice was also eliminated using this approach (28).

We also observed that G6F1 NOD.129/J backcross control female mice were completely protected from diabetes (12 of 12) at 25 wk of age (data not shown), a time at which the incidence of diabetes is >80% in female NOD mice in our colony. This result was expected due to the colocalization of the Idd4 locus and the MIP-1α gene on chromosome 11. Since a large region of 129/J-derived genes on chromosome 11 probably confers resistance to diabetes, the NOD.129/J strain may be a less appropriate control for the NOD.MIP-1α−/− mice than the NOD.MIP-1α+/− strain. Analyses of NOD.MIP-1α+/− mice enabled us to examine the possibility that Idd4 derived from the NOD strain interfered with the assessment of diabetes in NOD.MIP-1α−/− mice. These results provide further evidence that MIP-1α plays a unique effector role in the pathogenesis of type I diabetes in NOD mice.

To examine the changes in chemokine profiles in the pancreas of female NOD.MIP-1α−/− and NOD.MIP-1α+/− mice and their correlation to the onset of diabetes, intrapancreatic chemokine concentrations were determined in whole tissue homogenates by ELISA. MIP-1α was not detected in 15- to 20-wk-old nondiabetic and diabetic NOD.MIP-1α−/− mice (Fig. 7D). While an average of about 400 pg/mg tissue of MIP-1β was found in the pancreata of nondiabetic NOD.MIP-1α−/− mice, MIP-1β was not detected in diabetic NOD.MIP-1α−/− mice (Fig. 7D). No significant differences in MCP-1 concentrations were observed in the pancreas of recent onset diabetic and nondiabetic NOD.MIP-1α−/− mice. Levels of intrapancreatic chemokines in 15- to 20-wk-old nondiabetic and diabetic wild-type NOD mice (Fig. 2). Thus, while the absence of MIP-1α reduces insulitis and diabetes incidence in NOD.MIP-1α−/− mice, impaired expression of MIP-1β in the pancreas is characteristic of NOD.MIP-1α−/− mice that progress to overt diabetes.

Discussion

Our results demonstrate that the temporal patterns of differential expression of MIP-1α, MIP-1β, and MCP-1 in the pancreas are associated with either the progression or the prevention of insulitis and spontaneous type I diabetes. We found that early pancreas inflammation (≤10 wk of age) correlates with a high relative expression of MIP-1α in NOD mice. During this stage, NOD islets are infiltrated by both APCs and T cells, and this infiltration is
accompanied by the subsequent expression of proinflammatory Th1 cytokines associated with an invasive insulitis (26). By comparison, NOR islet infiltrates are composed primarily of APCs and relatively few, if any, T cells at this time. Our findings that pancreatic MIP-1α levels are high and MIP-1β levels are low during early islet infiltration in NOD mice may partially explain the differences in composition of the NOD and NOR islet cellular infiltrates.

The transgenic expression of MCP-1 in islet β cells yields an insulitis characterized by a nondestructive peri-islet monocyte infiltrate (29). During the early stages of islet infiltration in NOD and NOR mice, MCP-1 expression by pancreas-resident cells may contribute to the recruitment of a first wave of mononuclear cells composed mainly of APCs with a consequent attraction of lymphocytes. The outcome of this inflammatory response may depend on the presence of other chemokines, such as MIP-1α and MIP-1β. In this manner early intrapancreatic expression of CC chemokines may determine the quality of the islet infiltrate as destructive insulitis develops in female NOD mice. Differences in the severity of insulitis between NOD and NOR mice may also be regulated by genetically determined factors, such as the ability of resident APCs to interact with autoreactive T cells and modulate the profiles of intraislet cytokines and chemokines. Indeed, NOD.Scid mice, which do not have functional T or B cells, express detectable levels of MCP-1 but neither MIP-1α nor MIP-1β in the pancreas, suggesting that the intrapancreatic expression of MIP-1α and MIP-1β depends on the presence of infiltrated mononuclear cells.

An increase in the intrapancreatic MIP-1α:MIP-1β ratio was also noted in recent onset diabetic NOD female mice relative to their nondiabetic littermates. This pattern of chemokine content correlates with the presence of a Th1-enriched environment in the pancreas at diabetes onset (30–32), consistent with the presence of a higher IFN-γ:IL-4 concentration ratio in the pancreata of diabetic female NOD mice than in nondiabetic NOD mice (31, 32). Collectively, these findings implicate a temporal relationship between a higher intrapancreatic MIP-1α:MIP-1β ratio and the development of destructive insulitis and overt diabetes in NOD mice. Thus, although MIP-1α and MIP-1β are highly related proteins, they may have opposing functions during autoimmune inflammation (33–35).

Our previous finding that IL-4 protects NOD mice from diabetes (18) was informative about the biological relevance of the chemokine profiles observed in unmanipulated NOD pancreata. Most importantly, we found that the MIP-1α:MIP-1β plus MCP-1 ratio in the pancreas was reduced in IL-4-treated compared with control NOD mice. MIP-1α can stimulate macrophage secretion of IL-1α, IL-6, and TNF-α, which may promote NO production by macrophages and induce Fas-mediated apoptosis of islet β cells (36, 37). MIP-1α also preferentially attracts CD8+ T cells, which are required for the development of diabetes and are a source of MIP-1β in vivo (34, 38, 39). Thus, inhibition of MIP-1α by IL-4 may help protect islet β cells from attack by effector CD8+ T cells. In addition, since IL-4 can suppress MIP-1α secretion by stimulated monocytes (19), IL-4 treatment of NOD mice may inhibit the production of MIP-1α by monocytes/macrophages and/or islet-derived endothelial cells, thereby reducing early islet infiltration. Finally, the results obtained with IL-4 treatment suggest a mechanism of Th2 cell-mediated protection of NOD mice from diabetes (18). Thus, the level of MIP-1α expression is down-regulated in the spleen and pancreas of IL-4-treated mice consistent with its association with Th1 responses (4–7).

Previous studies have shown that in vivo neutralization of the activity of MIP-1α prevented EAE, while the neutralization of MIP-1β significantly exacerbated the disease (5, 33). In contrast, we found that anti-MIP-1β treatment not only did not accelerate the development of diabetes in NOD.Scid recipients of transferred diabeticogenic T cells but, rather, provided a small amount of protection of these recipients. This may have resulted from the high autoreactivity of the T cells from diabetic mice and their ability to rapidly transfer disease in this acute model. Indeed, increased MIP-1β levels in the pancreas are associated with IL-4-mediated protection from diabetes only at 10 wk of age. The latter finding is consistent with our recent report that the transfer of regulatory T cells induced by oral insulin treatment of NOD mice elicits a nondestructive insulitis characterized by a MIP-1β-enriched environment in the pancreas of NOD.Scid recipients (40). Inasmuch as an increased expression of MIP-1β occurs only in the pancreata of IL-4-treated NOD mice, MIP-1β may mediate the down-regulation of Th1 cell responses in the pancreas, but not secondary lymphoid organs (e.g., spleen). This idea is also reflected by our finding that the relative amount of MIP-1β is high in the pancreas of diabetes-free NOD.MIP-1α−/− mice compared with that in diabetic NOD.MIP-1α−/− mice. Our results support the ideas that a higher ratio of IL-4 plus MIP-1β:IFN-γ plus MIP-1α in the pancreas affords protection from diabetes and that islet-infiltrated T cells are probably the primary source of MIP-1α and MIP-1β in the pancreas.

The intrapancreatic content of MCP-1 increased in 10- and 20-wk-old NOD mice treated with IL-4. This is an interesting finding, since our analyses of NOD.Scid mice demonstrated that MCP-1 expression does not depend on pancreas inflammation or the presence of B and T cells. C57BL/6 mice (diabetes-resistant) between 5 and 30 wk of age do not express MIP-1α, MIP-1β, or MCP-1 in the pancreas, demonstrating the importance of the NOD/NOR genetic background in pancreas chemokine expression (our unpublished observations). MCP-1 and IL-4 are expressed coordinately during several immune responses, and MCP-1 blocks the adoptive transfer of EAE (4, 7). An increase in mucosal MCP-1 elicited by an oral autoantigen regulates tolerance induction by down-regulating Th1 responses and increasing IL-4 in EAE (41). IL-4 may induce a Th2 environment and the production of MCP-1 in pancreatic islets, particularly by endothelial cells (42). Indeed, our detection of differences in MCP-1 production in whole pancreata but not islet T cell infiltrates is compatible with the idea that the source of increased MCP-1 following IL-4 treatment may be islet-resident cells.

Th1 and Th2 cells differentially express chemokine receptors and migrate in response to different chemokines. The selective down-regulation of splenic T cell and intrapancreatic CCR5 mRNA may be significant due to its association with Th1 immune responses (9–11). Accordingly, reduced CCR5 mRNA in splenic T cells and islet-infiltrated cells after IL-4 treatment may reflect diminished Th1 activity. It is also possible that reduced CCR5 message in islets merely reflects the lower levels of T cell infiltration in IL-4-treated mice. We believe that the reduction in CCR5 mRNA expression is not solely due to a general reduction in insulitis afforded by IL-4 treatment, since the levels of expression of several other chemokine receptors, i.e., CCR1, CCR2, CCR3, CCR4, CXCR3, and CXCR4, remain unaltered, and an identical reduction in the level of CCR5 mRNA was observed in splenic T cells from IL-4–treated mice. In addition, we observed similar selective decreases in CCR5 expression in the infiltrated islets of 10-wk-old NOR mice (our unpublished observations), which, as discussed above, exhibit a intrapancreatic profile of MIP-1α, MIP-1β, and MCP-1 expression similar to that in IL-4–treated NOD mice.

Our findings that neutralization of MIP-1α in NOD.Scid mice upon adoptive transfer of diabeticogenic T cells and that deficient
expression of MIP-1α in NOD mice protects them from destructive insulitis and diabetes onset suggest that MIP-1α profoundly influences the pathogenesis of type I diabetes in NOD mice. Since MIP-1α may both influence the quality of the islet infiltrate and act as a costimulatory factor (Refs. 2, 39, and 43 this report), a deficiency in MIP-1α may establish a higher threshold for the activation and expansion of autoreactive T cells and other effector cells, such as macrophages. In this regard it is noteworthy that increases in the level of MIP-1α as well as the number of T cells expressing the CCR5 receptor occur in the CNS of patients with active multiple sclerosis (44). Thus, interactions between MIP-1α and CCR5 may contribute to the pathogenesis of inflammatory lesions in several T cell-mediated autoimmune diseases.

In conclusion, our findings provide in vivo evidence for a role of certain CC chemokines and CC chemokine receptors in the development and pathogenesis of type I diabetes. MIP-1α and MCP-1 play an early role in the recruitment of mononuclear cells to the islet and the establishment of insulitis in NOD mice. Young NOD mice exhibit a decreased intrapancreatic MIP-1α: MIP-1β plus MCP-1 ratio consistent with their diabetes resistance. A close correlation exists between Th2 cytokine responses, a high MIP-1β plus MCP-1:MIP-1α chemokine ratio, and reduced CCR5 expression elicited following IL-4 treatment in the pancreas of NOD mice protected from diabetes. Disruption of MIP-1α expression in NOD mice prevents and delays the onset of overt disease, an observation that can be explained in part by a reduction in destructive insulitis in these mice. There is growing evidence that the interrelationship of cytokines, chemokines, chemokine receptors, and adhesion molecules determines the extent of leukocyte migration to and the nature of inflammation at different target sites (1 and 44–46 and this report). Further experimentation is required to determine the chemokine and chemokine receptor interactions as well as the phenotypes of islet infiltrated cells in the pancreas of NOD-MIP-1α−/− mice. Temporal modulation of CC chemokines and their interactions with CC chemokine receptors may represent a novel therapeutic approach in the prevention of destruction of insulin and spontaneous type I diabetes.

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References


