Dendritic Cells and Macrophages Are the First and Major Producers of TNF-α in Pancreatic Islets in the Nonobese Diabetic Mouse

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Dendritic Cells and Macrophages Are the First and Major Producers of TNF-α in Pancreatic Islets in the Nonobese Diabetic Mouse

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The nonobese diabetic (NOD) mouse spontaneously develops autoimmune insulin-dependent diabetes mellitus (IDDM) and serves as an animal model for human type I diabetes. TNF-α is known to be produced by islet-infiltrating mononuclear cells during insulitis and subsequent β cell destruction and has been implicated in the pathogenesis of IDDM. Previously, T cells have been suggested as the main source of TNF-α in the islet infiltrate. However, on immunohistochemical analysis of TNF-α expression in islets, we are able to show that the staining pattern of TNF-α resembles that of dendritic cells (DC) and macrophages (Mφ) rather than T cells and that TNF-α is expressed in islets at the very early stages of insulitis when no T cells are detected. On double staining for TNF-α and cell surface markers, we can demonstrate that TNF-α staining clearly correlates with DC and Mφ, whereas there is a poor correlation with T cells. This feature was observed at both early and late stages of insulitis. TNF-α expression was also seen in NOD-SCID islets, in addition to a peri-islet infiltration consisting of DC and Mφ, indicating that T cells are not required for the early DC and Mφ infiltration and TNF-α expression in islets. In conclusion, our results show that DC and Mφ are the major, early source of TNF-α in the NOD islet infiltrate and that TNF-α can be expressed independently of T cells, indicating that the early DC and Mφ infiltration and expression of TNF-α are crucial in initiation of diabetes.

The nonobese diabetic (NOD) mouse spontaneously develops autoimmune IDDM (1) and serves as an animal model for human type I diabetes. The processes leading to the autoreactive inflammation in the pancreas and subsequent β cell destruction are not completely understood, but Mφ (2, 3) as well as CD4+ and CD8+ T cells (2, 4, 5) are required for the development of the disease and cytokines such as IFN-γ, IL-1, and TNF-α (6) are implicated in playing a role in disease development. TNF-α is a pleiotropic cytokine known to be produced by islet-infiltrating mononuclear cells during insulitis in the pancreas. TNF-α serves as a proinflammatory cytokine and affects several aspects of the inflammatory process, including chemotaxis and activation of leukocytes (7), up-regulation of adhesion molecules on endothelial cells (8–10), and maturation and migration of dendritic cells (DC) (11). In addition, TNF-α, in combination with other cytokines such as IL-1 and IFN-γ, has been shown to exert β cell cytotoxicity in vitro by inducing apoptosis (12–14). Thus TNF-α may act directly and indirectly in the autoimmune processes mediating β cell destruction. However, the role of TNF-α in disease development is still unclear, since treatment of NOD mice with rTNF-α or blocking anti-TNF-α Abs may either prevent or exacerbate disease, depending on the age of the mice. Thus, Yang et al. have shown that treating newborn or 2-wk-old female NOD mice with rTNF-α for 3 wk leads to an earlier onset and a higher incidence of diabetes, whereas treating them with anti-TNF-α from birth prevents disease development (15). In contrast, if TNF-α treatment is initiated at 4 wk of age, onset is delayed (15). In addition to this, a dramatic reduction in development of diabetes is seen in long term (4 mo) treatment with TNF-α when treatment is started at 10 wk of age (15, 16). Similar results have been obtained by Jacob and coworkers (17), who were able to show that treatment of 8-wk-old NOD mice with rTNF-α for 4 or 8 wk reduced the incidence of insulitis, whereas treatment with anti-TNF-α for 8 wk resulted in an increased incidence. In addition to these findings, the important role played by TNF-α in diabetes development is further demonstrated in p55 TNF-R transgenic NOD mice, which constitutively express soluble neutralizing receptors. In these mice, both insulitis and diabetes development are inhibited (18). Furthermore, the expression of transgenic TNF-α in the β cells of normal mice (19, 20) or diabetes-prone NOD mice (21) results in a massive insulitis but does not lead to development of diabetes. The protective effect of TNF-α observed in TNF-α transgenic NOD mice appears to result from prevention of development of islet-specific autoreactive T cells and may be explained by the fact that TNF-α fails to be expressed in young mice and is only expressed in adults from 7 wk of age. In summary, TNF-α appears to exacerbate disease in young NOD mice, whereas it is protective in old mice.

To date, the source of TNF-α in the islet infiltrate has been suggested to be the T cell (22–24), whereas expression of TNF-α by DC and Mφ in the islet infiltrate has not been shown. In fact, it has been suggested that Mφ produce very little TNF-α in the islet infiltrate (22, 23). However, we are able to show that the TNF-α staining in the islets of Langerhans exhibits a dendritic morphology and resembles the staining pattern observed for DC and Mφ and that TNF-α is detectable in the islets in the very early stages of insulitis, when DC and Mφ, but no T cells are detected, suggesting that DC/Mφ may be a source of TNF-α in the islet infiltrate. On double staining for TNF-α and cell surface markers,
we demonstrate that TNF-α staining clearly correlates with the DC marker N418 as well as the Mφ markers F4/80 and Mac-1, whereas a poor correlation is observed with the T cell markers CD4 and CD8. DC and Mφ infiltration and expression of TNF-α occur independently of T cells, as these processes can be observed in young NOD mice in the absence of infiltrating T cells as well as NOD-SCID mice lacking T cells. In conclusion, our results indicate that DC and Mφ, rather than T cells, are the major source of TNF-α in the islet infiltrate; that DC and Mφ infiltration and TNF-α expression may occur independently of T cells; and that the early APC-associated TNF-α may be important for the initiation and/or development of diabetes.

Materials and Methods

Animals and pancreatic tissue sampling

Female NOD and NOD-SCID mice were purchased from Bommenice (Bommeniðt Gård Breeding and Research Center, Ry, Denmark) and were used at the ages of 4 to 18 wk of age, as indicated. For pancreatic tissue sampling, mice were sacrificed by cervical dislocation, pancreata were removed and frozen in 2-methyl butane (KEBO Lab, Lund, Sweden), chilled on liquid nitrogen, and then stored at −70°C for sectioning.

Immunohistochemical and immunofluorescence staining

Levels of mononuclear cell infiltration in islets of Langerhans were determined in NOD mice at various ages. Cryosections (8 μm thick) were allowed to dry in air overnight and were subsequently fixed with acetone (Histolab Products, Västra Frölunda, Sweden) at −20°C for 10 min. Endogenous biotin and avidin binding activity was blocked with an Avidin/Biotin Blocking Kit (Vector Laboratories, Burlingame, CA) for 15 min. Sections were incubated with primary Abs directed toward N418 (5 μg/ml, Serotec, Oxford, U.K.), F4/80 (1:10, Serotec), Mac-1 (5 μg/ml, Boehringer Mannheim, Mannheim, Germany), CD3 (5 μg/ml, PharMingen, San Diego, CA), CD4 (5 μg/ml, PharMingen), and CD8 (5 μg/ml, PharMingen) for 1 h. Sections were then incubated with the appropriate biotin-conjugated secondary Abs, i.e., goat anti-Armenian hamster IgG (1:500, Jackson ImmunoResearch Laboratories, West Grove, PA), for detection of N418 and goat anti-rat IgG (1:400, Jackson ImmunoResearch Laboratories) for detection of F4/80, Mac-1, CD4, or CD8 for 30 min, followed by incubation with HRP-conjugated streptavidin (ABC-HRP, 1:110, Vector Laboratories) for 30 min. Finally, the sections were incubated with 0.5 mg/ml diaminobenzamidine (DAB, Saveen Biotech, Malmo, Sweden) diluted in Cy3-labeled goat anti-rat IgG (10 μg/ml, Amersham Life Science) for 30 min, followed by 1% normal rat serum for 30 min, and then anti-TNF-α overnight. Finally, sections were incubated with FITC-labeled mouse anti-rat IgG1 for 1 h. Slides were mounted with Dako Fluorescence Mounting Medium (Dako, Glostrup, Denmark).

Image analysis

DAB staining was examined with a Leitz Aristoplan light microscope (Leica, Göteborg, Sweden) equipped with a PC-based Quantimet Q 500 image analysis program (Leica). Quantification of levels of infiltration by cells expressing the N418, F4/80, Mac-1, CD4, or CD8 cell surface markers, as well as expression of TNF-α in the islets, was achieved by setting

![Image 337x97 to 529x562](Image 337x97 to 529x562)

**FIGURE 1.** Kinetics of mononuclear cell infiltration and TNF-α expression in the islets of Langerhans in the NOD mouse. Pancreatic sections from 4-, 6-, 10-, 14-, and 18-wk-old mice were stained for: TNF-α (A); N418, F4/80, and Mac-1 (B); and CD4 and CD8 (C). Percentage of area stained was determined by the image analysis system Quantimet Q 500, and results are shown as mean percentage stained area ± SEM.
an interval of brown staining (DAB), which was detected as positive by the computer, whereas the blue hematoxylin counterstaining was ignored. The percentage of stained area within each islet was determined for ~40 different islets in five animals per age group. To analyze the degree of TNF-α and cell surface marker staining, pancreatic sections from 14-wk-old NOD mice were stained for indicated markers and visualized by DAB. Representative parallel sections are shown stained for: a, TNF-α, b, N418, c, F4/80, d, Mac-1, e, CD4, and f, CD8.

Table I. Number and percentage of islets with detectable infiltration by cells expressing the indicated markers

<table>
<thead>
<tr>
<th>Marker</th>
<th>No. Infiltrated Islets/Total No. of Islets</th>
<th>% Infiltrated Islets</th>
</tr>
</thead>
<tbody>
<tr>
<td>TNF-α</td>
<td>53/53</td>
<td>100%</td>
</tr>
<tr>
<td>N418</td>
<td>34/37</td>
<td>92%</td>
</tr>
<tr>
<td>F4/80</td>
<td>42/45</td>
<td>93%</td>
</tr>
<tr>
<td>Mac-1</td>
<td>40/44</td>
<td>91%</td>
</tr>
<tr>
<td>CD4</td>
<td>6/42a</td>
<td>14%</td>
</tr>
<tr>
<td>CD8</td>
<td>5/44b</td>
<td>11%</td>
</tr>
</tbody>
</table>

a The number of islets with detectable CD4 T cell infiltration/total number of islets examined in five 4-wk-old NOD mice were 3/7, 0/8, 0/5, 3/13, and 0/9, respectively.

b The number of islets with detectable CD8 T cell infiltration/total number of islets examined in five 4-wk-old NOD mice were 2/6, 0/8, 3/12, and 0/10, respectively.

The kinetics and staining pattern of TNF-α in the NOD islet infiltrate resembles that seen for DC and MΦ

On immunohistochemical analysis of pancreata from NOD mice, TNF-α is detectable during the very early stages of insulitis, i.e., at 4 wk of age. At this age, DC and MΦ are detectable in the islets, whereas T cells are undetectable in the majority of islets (Fig. 1, Table I). In 3/5 four week old animals, the islets lacked detectable T cell infiltration, whereas in two animals CD4+ T cells could be detected in 3/7 and 3/13 islets respectively, and CD8+ T cells could be detected in 2/6 and 3/12 islets respectively. TNF-α is continuously expressed in the islets throughout the course of insulitis development from at least 4 wk of age and the level of expression increases with increasing mononuclear cell infiltration (Fig. 1). Thus, the kinetics of TNF-α expression in the islet infiltrate appears to correlate more closely with the infiltration kinetics of DC and MΦ rather than T cells.

Moreover, we are able to show that the morphology of the TNF-α staining in the islet infiltrate (Fig. 2a) strongly resembles a DC/MΦ staining pattern, with thin elongated processes or dendrites and irregularly shaped cell bodies, a staining pattern which is very similar to the pattern observed when staining with antibodies toward the DC marker N418 and the MΦ markers F4/80 and Mac-1 (Fig. 2, b–d, respectively). A similar TNF-α staining pattern is observed in NOD islets from mice at all ages, ranging from 4 to 18 wk of age, the only difference being that the level of TNF-α expression increases with age. Similarly, TNF-α associated with T lymphocytes would be expected to stain with a discrete staining pattern as can be seen when staining for CD4 or CD8 (Fig. 2, e and f, respectively), but TNF-α staining with this morphology cannot be observed. The early presence of TNF-α, at 4 wk of age, as well as its dendritic appearance in staining suggests that DC and MΦ rather than T cells could be a main source of TNF-α in the NOD islet infiltrate.

**Results**

The kinetics and staining pattern of TNF-α in the NOD islet infiltrate resembles that seen for DC and MΦ

To investigate whether DC and MΦ, rather than T cells, were the main source of TNF-α in the NOD islet infiltrate, we performed double immunofluorescence staining where staining for TNF-α with FITC-labeled Abs was combined with staining for the cell surface Ags N418, F4/80, Mac-1, CD4, and CD8 with Cy3-labeled Abs. In Figure 3, parallel sections of representative islets from 4- and 14-wk-old animals stained for TNF-α and the various cell surface markers are shown. Figure 3, a and g, shows that the TNF-α staining in islets from 4- and 14-wk-old mice, respectively, have a similar dendritic staining pattern, although levels of TNF-α expression are increased in 14-wk-old mice compared with 4-wk-old mice. It can be seen that TNF-α expression correlates very well...
with the DC marker N418 (Fig. 3, b and h) as well as with the Mφ markers F4/80 (Fig. 3, c and i) and Mac-1 (Fig. 3, d and j) in both young and old NOD mice. In contrast to this, there is a poor correlation of TNF-α with CD4+ (Fig. 3, e and k) and CD8+ T cells (Fig. 3, f and l). Note that the localization as well as the morphology of the TNF-α staining is similar to that observed for DC and Mφ, but differs from that seen for T cell staining. Islets are normally surrounded by TNF-α-expressing cells, and in addition to this, a few TNF-α-expressing cells with dendritic morphology can be seen in intra-islet positions (Fig. 3a). A similar distribution is seen for Mφ and DC. Mφ are usually found in peri-islet locations, whereas DC are found in peri-islet as well as in intra-islet locations, close to the front of β cell destruction and at the site of T cell infiltration. T cells are mainly intra-islet, often with TNF-α positive dendritic structures in close proximity. The correlation of TNF-α with DC and Mφ was seen at both 4 and 14 wk of age, the

FIGURE 3. Correlation of TNF-α with cell surface markers. Sections of pancreata from 4 (a–f) and 14-wk-old (g–l) NOD mice were stained by double immunofluorescence for TNF-α (FITC) and cell surface markers (Cy-3). Representative parallel sections stained for TNF-α only (a, g), and TNF-α in combination with the cell surface markers N418 (b, h), F4/80 (c, i), Mac-1 (d, j), CD4 (e, k), and CD8 (f, l) are shown. Arrows indicate cells staining double positive for both TNF-α and the cell surface marker.
staining pattern being similar except that T cells were only rarely detected in islets of 4-wk-old mice. The percentage of TNF-α associated with the different cell surface markers was quantified by image analysis (Fig. 4). It was found that most of the TNF-α expressed in the islet infiltrate correlated with the cell surface markers N418, F4/80, or Mac-1. Thus, in 4-wk-old mice, 37% of the TNF-α expressed in the islet infiltrate correlated with the N418 marker, 50% with F4/80, and 16% with Mac-1. In 14-wk-old mice, the corresponding figures were 41% for N418, 46% for F4/80, and 26% for Mac-1. A certain degree of correlation between TNF-α and the CD4 and CD8 cell surface Ags could also be observed (1.6% for CD4 and 0.2% for CD8 in 4-wk-old mice and 20% for CD4 and 17% for CD8 in 14-wk-old mice). However, most of this correlation appeared to be due to TNF-α-expressing DC/Mφ overlapping with CD4⁺ or CD8⁺ T cells in the islet infiltrate, especially in highly infiltrated islets. In Figure 3, k and l, dendritic-like structures, which appear double stained, can be seen in very close proximity to T cells. As judged by the staining morphology, these TNF-α-expressing cells are probably DC/Mφ rather than T cells, and therefore the true correlation between TNF-α and T cells may be overestimated by image analysis. In addition, a certain proportion of these cells may be DC that are expressing CD4 or CD8 (25,
26). When primary Abs were substituted for their relevant isotype control Abs in the double immunofluorescence staining method described in Materials and Methods, no staining could be detected (data not shown). Substitution of anti-TNF-α Ab by rat IgG1 on sections, after staining for the cell surface Ags, resulted in staining for the cell surface markers alone (data not shown); and when preincubating the anti-TNF-α Ab with rTNF-α before adding it to the sections, staining was prevented (data not shown).

It is clear that there is a certain degree of overlap between the three DC/MΦ surface markers used. Thus, we investigated whether the major part of the TNF-α staining observed in the islet infiltrate was associated with the total DC/MΦ population or whether other cell types could also account for a proportion of the TNF-α staining observed. To this end, pancreatic sections from 4- and 14-wk-old animals were double stained for TNF-α (FITC) and a cocktail of Abs directed to the DC/MΦ surface markers N418, F4/80, and Mac-1 (Cy-3). The vast majority of the TNF-α expressed in the islet infiltrate is contained within the DC/MΦ population, as indicated by the double staining shown in yellow (Fig. 5). On image analysis of the sections, it was found that 70 ± 2.9% and 78 ± 2.2% (mean ± SEM) of the TNF-α expressed in the islet infiltrate correlated with the DC/MΦ markers in 4- and 14-wk-old NOD mice, respectively. The TNF-α that did not correlate with these cell surface markers had the same dendritic morphology, suggesting that it may be produced by DC/MΦ, which are not detectable by the markers used in this study.

Initial DC and MΦ infiltration and TNF-α expression is independent of T cells

To confirm that DC/MΦ infiltration in the islets of Langerhans is a feature specific for diabetes development, we stained pancreatic sections of control 8-wk-old C57BL/6 mice for the DC marker N418 and the MΦ marker F4/80. Low numbers of DC/MΦ could be observed in the exocrine pancreas, whereas islets lacked DC/MΦ infiltration (Fig. 6, a and b), suggesting that DC/MΦ infiltration in the endocrine pancreas is a feature of diabetes development and occurs only in mice of a diabetes prone background.

We next wanted to investigate the T cell dependence of DC/MΦ infiltration and TNF-α expression in pancreatic islets. We have been able to show that expression of TNF-α in early stages (undetectable T cell infiltration in most islets) as well as late stages...
(profound T cell infiltration) of insulitis correlates well with the DC/Mφ population, suggesting that these cells could be important producers of TNF-α in the islet infiltrate. However, T cells have been shown to produce TNF-α in the late stages of insulitis and β cell destruction (22–24), and although we find poor correlation between TNF-α and T cell markers, it could be argued that the TNF-α found to correlate with DC/Mφ could be exogenously produced, for example by T cells, and then taken up by DC/Mφ expressing TNF-R. DC/Mφ are detectable histochemically in islets before T cells, and it has been suggested that DC/Mφ are the first cells to infiltrate the islets and therefore initiate the autoreactive process (27–29). However, PCR analysis of TCR Vβ elements has shown that T cell clones are present in the islets of Langerhans as early as 2 wk of age (30), suggesting that β cell-specific T cells initiate the disease.

To investigate whether DC/Mφ are able to infiltrate islets and express TNF-α in the absence of T cells, pancreatic sections from 8-wk-old NOD-SCID mice, which lack T cells, were stained for a panel of cell surface Ags and TNF-α. Indeed, infiltration of N418- and F4/80-positive cells, as well as TNF-α expression, was detected in islets from NOD-SCID mice (Fig. 7, a–c), demonstrating that in the early stages of insulitis, DC/Mφ infiltration occurs independent of T cells. However, it was noted that levels of DC/Mφ infiltration and TNF-α expression were lower in NOD-SCID mice than in age-matched NOD mice (Fig. 7, a–f), suggesting that the presence of T cells accelerates the development of the DC/Mφ infiltration. Thus, our results strongly suggest that whereas T cells are required for the development of a massive insulitis and subsequent β cell destruction, initiation of insulitis can occur in the absence of T cells. No T cell infiltration, as determined by CD3+ CD4+, and CD8+ lymphocytes, could be detected in islets of either NOD-SCID mice or C57BL/6 mice, whereas it was detectable in NOD islets (data not shown).

**Discussion**

In the present study, we have been able to show that local expression of TNF-α in the NOD islet infiltrate at both the early and late stages of insulitis correlates well with DC and Mφ markers, whereas a poor correlation with T cells is observed, suggesting that DC/Mφ are an important source of TNF-α in the islet infiltrate. In previous publications, Held et al. (22) and Toyoda et al. (23) studied TNF-α mRNA expression by islet-infiltrating cells by in situ hybridization and RT-PCR, respectively, and concluded that CD4+ T cells and not Mac-1+ macrophages are the source of TNF-α in islets. These results are in contrast to our own, but the contradictions may be explained by several differences in the experimental settings. Whereas we have studied the expression of TNF-α protein by immunohistochemistry and immunofluorescence in islets, previous investigators have studied the expression of TNF-α mRNA by in situ hybridization or RT-PCR. In addition, we studied the expression of TNF-α protein over a broad time period, from 4 wk to 18 wk of age, whereas previous investigators studied TNF-α mRNA expression in older animals, aged 12–18 wk.
it could be argued that the TNF-α protein found to correlate with the DC/Mϕ population could be produced by other cell types, for example T cells, and then taken up by DC/Mϕ via TNF-R. However, as TNF-α protein expression can be detected in islets of 4-wk-old NOD mice lacking a detectable T cell infiltration, as well as in NOD-SCID islets, it is likely that, at least in young animals, TNF-α is produced by islet-infiltrating DC/Mϕ. Another possibility is that production of other cytokines, such as IL-1, by DC or Mϕ may induce β cells to produce TNF-α, which would then be taken up by islet-infiltrating DC and Mϕ. IL-1-induced TNF-α production by the β cell line βTC1, as well as by mouse β cells, has been demonstrated in vitro (31, 32).

Furthermore, on phenotypic analysis of TNF-α-expressing cells, we have investigated the correlation of TNF-α and mononuclear cell markers in situ on pancreatic sections from NOD mice, whereas previous studies were performed on mononuclear cells purified from pancreata by collagenase digestion and subsequently sorted by FACS. When purifying islet-infiltrating cells in this manner, it is probable that mainly intra-islet-infiltrating cells are obtained, whereas there is the risk of losing cells at peri-islet locations, which is where most of the F4/80+ and Mac-1+ Mϕ are situated. In addition, DC/Mϕ are more sensitive to handling than lymphocytes, and therefore intra-islet DC/Mϕ may be underrepresented. This may explain the low numbers of TNF-α mRNA-expressing Mϕ observed in these investigations. Finally, we have been using three different markers to detect DC/Mϕ, whereas previous investigators used only Mac-1, which is the DC/Mϕ marker found to have the lowest correlation with TNF-α in this study.

The aim of this study was to investigate the source of TNF-α in the islet infiltrate. With the methods used in this study, it is difficult to define the role of TNF-α in the development of insulitis and diabetes. However, the pathogenic role of TNF-α at the early stages of diabetes development has been clearly demonstrated. Treatment of newborn or 2-wk-old NOD mice with rTNF-α leads to an earlier onset and a higher incidence of diabetes, whereas treatment with anti-TNF-α Abs from birth prevents disease (15), thus supporting our claim that early production of TNF-α is crucial to disease development. Furthermore, in p55 TNF-R transgenic NOD mice that constitutively express soluble neutralizing p55 TNF receptors, diabetes development is inhibited (18). The precise role of TNF-α in the islets at these early time points is not known, but TNF-α, in combination with other cytokines such as IL-1, has been shown to be toxic to β cells (12–14); thus, early expression of TNF-α and other cytokines in islets may induce a partial β cell dysfunction or destruction facilitating the uptake of β cell Ags by APC. TNF-α is also known to be an important maturation factor for DC, inducing the up-regulation of MHC class I and II, CD40, CAM-1, participating in the recruitment of mononuclear cells into the islets (8, 9, 18). Thus, the correlation of TNF-α with APC demonstrated in this study may be important for the initiation of insulitis leading to the massive T cell-mediated β cell destruction and subsequently resulting in overt diabetes.

Moreover, we are able to show DC/Mϕ infiltration in the islets of NOD-SCID mice. The SCID mutation renders mice immunodeficient by preventing the normal rearrangement of TCR and Ig genes, resulting in a lack of mature T and B lymphocytes (36). NOD-SCID mice were generated by an initial CB17-SCID × NOD Lt cross followed by several backcrosses of +/SCID heterozygotes (37); NOD-SCID mice remain free from insulitis and IDDM throughout life (37–40). To investigate whether the early DC/Mϕ infiltration in islets is T cell dependent, we stained pancreatic sections from 8-wk-old NOD-SCID mice for a panel of cell surface markers and TNF-α and found peri-islet infiltration of cells expressing F4/80, N418, and TNF-α. These results showed that the early DC/Mϕ infiltration into the islets can occur independently of T cells but requires the NOD autoimmune background, as C57BL/6 islets lack DC/Mϕ infiltration, suggesting that DC/Mϕ and not T cells initiate the autoimmune inflammation and that the TNF-α expressed in islets of young mice is not T cell derived or T cell dependent.

Although we were unable to observe any T cell infiltration in the islets of the NOD-SCID mice used in this study, the leakiness that can be observed in some SCID mice must be considered. It has been shown, especially in older CB17-SCID mice, that despite the SCID mutation, clones of mature B and T lymphocytes may develop (41–44) and that the frequency of these cells has been shown to increase with age (42, 44). Thus, in a report by Bosma et al., evidence for mature B cells could be found in 2.5 to 5.4% of 3- to 4-mo-old CB17-SCID mice, and in 14- to 15-mo-old mice this figure had increased to 32.5% (44). In a later report by Nonoyama et al., it was demonstrated that varying levels of leakiness could be observed in 79% of 2- to 3-mo-old CB17-SCID mice (43). However, the leakiness of SCID mice has been shown to be strain dependent, and it is much lower in C3H-SCID (43) and in NOD-SCID mice (40). Indeed, a very low level of T and B cell development has been demonstrated in NOD-SCID mice, with only 2 of 30 mice, age 100 to 200 days, having detectable serum Ig, whereas Ig could be detected in the serum from 21 of 29 CB17-SCID mice at the same age (40). Furthermore, no CD3+ cells could be detected in the spleen of these mice, and islets remained free from lymphocyte infiltration throughout life (40). Therefore, we conclude that the DC/Mϕ infiltration and expression of TNF-α observed in the islets of NOD-SCID mice is T cell independent.

What mediates DC/Mϕ recruitment and activation in islets is not clear. However, intrinsic features of pancreatic islets may contribute to this early recruitment and activation of APC. β Cell dysfunctions such as increased insulin responses to glucose (45, 46), increased basal levels of plasma glucagon (47), some degree of insulin resistance (46), and an increased number of large size islets (48) have been observed early in the disease process of NOD mice. In addition, primary destructive events due to a viral infection of the pancreas have been suggested (49, 50); any of these features may contribute to the early DC/Mϕ inflammation in NOD islets. Although these results suggest that initial DC/Mϕ infiltration into the islets of Langerhans can occur independently of T cells, it could clearly be seen that age-matched NOD mice exhibited a higher level of DC/Mϕ infiltration than NOD-SCID mice, arguing for a critical role of T cells in the development of a pronounced DC/Mϕ infiltration in islets as well as in subsequent β cell destruction. We conclude that the early DC/Mϕ infiltration and expression of TNF-α in NOD islets can occur independently of T cells and thus initiate insulitis development, but that a subsequent T cell infiltration accelerates the process of insulitis and is
required for extensive β cell destruction and overt diabetes development.

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References


