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Dendritic cells (DC) present Ag to naive T cells and are therefore pivotal in shaping immune responses. DC may either immunize or tolerize T cells. Humans with pancreatic islet autoimmunity at high risk for insulin-dependent diabetes mellitus (IDDM) present the opportunity to investigate DC in autoimmune disease. We compared DC phenotype and function in 12 euglycemic, asymptomatic IDDM relatives with islet autoimmunity and controls matched for age, sex, and MHC class II alleles. DC were generated from adherent peripheral blood cells by culture with granulocyte/macrophage-CSF and IL-4. The yield of DC was significantly lower in IDDM relatives than in controls. While the DC phenotype, HLA-DR⁺CD14⁺, was expressed by ≥90% of the cells generated from relatives and controls, the proportion of cells that expressed CD1a and the costimulator molecules CD80 (B7-1) and CD86 (B7-2) was significantly lower in IDDM relatives. In addition, B7-1 and B7-2 expression per cell was significantly lower in IDDM relatives. These phenotypic changes were accompanied by reduced stimulation of autologous CD4 cells by DC from IDDM relatives. Similar findings were obtained in three recently diagnosed IDDM patients. These findings indicate that impairment of DC phenotype and function is a marker of islet autoimmunity and are consistent with a role for impaired DC function in the pathogenesis of autoimmune disease. The Journal of Immunology, 1998, 161: 2629–2635.

Materials and Methods

Subjects

First-degree relatives of IDDM patients (five males, seven females) positive for circulating Abs to islet Ags (Table I) were sex-matched, closely age-matched, and completely HLA class II-matched with healthy volunteer controls from the Australian Bone Marrow Donor Registry who were negative for Abs to islet Ags. The mean ages of at-risk IDDM relatives (25.1 yr; range, 8–50) and the control subjects (34.0 yr; range, 20–47) were not significantly different (p = 0.1). Subsequently, three patients with newly diagnosed (<3 mo) IDDM on insulin treatment and with well-controlled blood glucose levels were sex-, age-, and HLA-matched for study with three healthy controls (Table II). Subjects were bled from a forearm vein between 8:30 and 9:30 a.m. The study was approved by the Institution Human Ethics Committee.

Markers of islet autoimmunity

Islet cell Abs were measured by indirect immunofluorescence with doubling dilutions of serum on frozen sections of pancreas from a blood group
Precipitation of [35S]methionine-labeled recombinant proteins synthesized by decarboxylase Abs and tyrosine phosphatase IA-2 Abs were measured by fluid phase 125I-labeled insulin-binding precipitation assay. Glutamic acid decarboxylase Abs and tyrosine phosphatase IA-2 Abs were measured by precipitation of [35S]methionine-labeled recombinant proteins synthesized after transcription-translation in the T7-coupled reticulocyte lysate system (Promega, Madison, WI). The four Ab assays have been included in all Immunology of Diabetes Workshops and Proficiency Programs (sponsored by Juvenile Diabetes Foundation International).

Culture medium and reagents
PBMC were cultivated in human tonicity RPMI 1640 containing 20 mM HEPES and 10% FCS. Recombinant human GM-CSF (rhGM-CSF) was purchased from PeproTech (Rocky Hill, NJ) or kindly provided by Dr. Nicos Nicola (The Walter and Eliza Hall Institute of Medical Research, Victoria, Australia). Recombinant human IL-4 (rhIL-4) was kindly provided by Schering Plough (Kenilworth, NJ).

Generation of DC from peripheral blood
PBMC were isolated from heparinized venous blood on Ficoll-Paque, resuspended in medium, and allowed to adhere to six-well plates (Costar, Cambridge, MA) coated with rat tail collagen. After 2 h at 37°C in 5% CO₂, the nonadherent cells were removed by a very gentle rinse, and the adherent cells were cultured for 7 days in medium containing 600 U/ml rhGM-CSF and 400 U/ml rhIL-4.

Flow cytometric analysis
Cells from all subjects were surface phenotyped by labeling with a panel of mouse mAbs (Table III) and analyzed on a FACScan (Becton Dickinson, Mountain View, CA). Cells were gated on forward and side scatter and by propidium iodide staining, and up to 10,000 cells with high forward and side scatter were counted. Data acquisition was performed with the FACScan Research Software, which automatically calculates mean fluorescence intensity (MFI) after setting markers on positive and negative peaks.

Table I. Characteristics of first-degree relatives at risk for IDDM

<table>
<thead>
<tr>
<th>Subject</th>
<th>Age</th>
<th>Sex</th>
<th>Alleles</th>
<th>Relationship to IDDM Relative</th>
<th>ICA (&lt;20 JDF U)</th>
<th>IAA (&lt;35 nU/ml)</th>
<th>GAD Ab (&lt;5 U)</th>
<th>IA-2 Ab (&lt;4 U)</th>
<th>FPPIR (mU/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>11</td>
<td>F</td>
<td>3,4,2</td>
<td>Sibling</td>
<td>63</td>
<td>82</td>
<td>75</td>
<td>15</td>
<td>93</td>
</tr>
<tr>
<td>2</td>
<td>48</td>
<td>F</td>
<td>3,4,2</td>
<td>Mother</td>
<td>160</td>
<td>92</td>
<td>89</td>
<td>14</td>
<td>117</td>
</tr>
<tr>
<td>3</td>
<td>44</td>
<td>F</td>
<td>3,12,2</td>
<td>Mother</td>
<td>160</td>
<td>-15</td>
<td>-4</td>
<td>-1</td>
<td>260</td>
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<td>4</td>
<td>8</td>
<td>F</td>
<td>4,8,48</td>
<td>Sibling</td>
<td>20</td>
<td>760</td>
<td>49</td>
<td>100</td>
<td>62</td>
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<tr>
<td>5</td>
<td>15</td>
<td>M</td>
<td>4,4,88</td>
<td>Sibling</td>
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<td>120</td>
<td>42</td>
<td>88</td>
<td>234</td>
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<tr>
<td>6</td>
<td>16</td>
<td>M</td>
<td>4,7,2,8</td>
<td>Sibling</td>
<td>25</td>
<td>3800</td>
<td>40</td>
<td>23</td>
<td>152</td>
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<td>7</td>
<td>18</td>
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<td>4,3,2</td>
<td>Sibling</td>
<td>160</td>
<td>30</td>
<td>4</td>
<td>88</td>
<td>41</td>
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<tr>
<td>8</td>
<td>16</td>
<td>M</td>
<td>3,3,2</td>
<td>Sibling</td>
<td>129</td>
<td>90</td>
<td>49</td>
<td>89</td>
<td>44</td>
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<td>9</td>
<td>46</td>
<td>F</td>
<td>4,4,88</td>
<td>Mother</td>
<td>43</td>
<td>100</td>
<td>11</td>
<td>11</td>
<td>28</td>
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<tr>
<td>10</td>
<td>50</td>
<td>F</td>
<td>3,4,2</td>
<td>Mother</td>
<td>16</td>
<td>200</td>
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</tr>
<tr>
<td>11</td>
<td>14</td>
<td>M</td>
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<td>Sibling</td>
<td>50</td>
<td>44</td>
<td>86</td>
<td>-7</td>
<td>36</td>
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<tr>
<td>12</td>
<td>18</td>
<td>M</td>
<td>4,4,7,8</td>
<td>Sibling</td>
<td>50</td>
<td>44</td>
<td>86</td>
<td>-7</td>
<td>36</td>
</tr>
</tbody>
</table>

* ICA, islet cell Abs; JDF, Juvenile Diabetes Foundation; IAA, insulin autoantibodies; GAD Ab, Abs to glutamic acid decarboxylase; IA-2 Ab, Abs to tyrosine phosphatase IA-2; FPPIR, first-phase insulin release, the sum of 1- and 3-min serum insulin concentrations after i.v. glucose injection; the threshold for the first percentile in a young adult population is 50 mU/L.

The yield of DC from IDDM relatives was reduced compared to controls (Table II). The presence of IL-4 was necessary for optimal yield of DC, as DC derived from monocytes in the presence of GM-CSF and IL-7 were recently described (17). The proportion of DC derived from IDDM relatives was reduced (Table II).

Table II. Properties of DC generated from patients with recently diagnosed IDDM

<table>
<thead>
<tr>
<th>Subject</th>
<th>Age</th>
<th>Sex</th>
<th>HLA DR DQ</th>
<th>Yield/10⁷ PBMC</th>
<th>% Positive</th>
<th>MFI</th>
<th>Mean ± SD × 10⁻³ cpm</th>
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</thead>
<tbody>
<tr>
<td>IDDM</td>
<td>40</td>
<td>F</td>
<td>3,4,2 8</td>
<td>5.0 ×10⁷</td>
<td>22</td>
<td>36</td>
<td>92 ± 80</td>
</tr>
<tr>
<td>Control</td>
<td>40</td>
<td>F</td>
<td>3,4,2 8</td>
<td>5.0 ×10⁷</td>
<td>22</td>
<td>36</td>
<td>92 ± 80</td>
</tr>
<tr>
<td>IDDM</td>
<td>32</td>
<td>M</td>
<td>3,4,2 8</td>
<td>5.5 ×10⁷</td>
<td>59</td>
<td>74</td>
<td>98 ± 91</td>
</tr>
<tr>
<td>Control</td>
<td>32</td>
<td>M</td>
<td>3,4,2 8</td>
<td>5.5 ×10⁷</td>
<td>59</td>
<td>74</td>
<td>98 ± 91</td>
</tr>
<tr>
<td>IDDM</td>
<td>40</td>
<td>M</td>
<td>3,4,2 8</td>
<td>8.0 ×10⁷</td>
<td>83</td>
<td>58</td>
<td>93 ± 99</td>
</tr>
<tr>
<td>Control</td>
<td>40</td>
<td>M</td>
<td>3,4,2 8</td>
<td>8.0 ×10⁷</td>
<td>83</td>
<td>58</td>
<td>93 ± 99</td>
</tr>
</tbody>
</table>

* Allo, allogeneic MLR; Auto, autologous MLR; 10,000 or 2,000 is the number of DC present in the allogeneic or autologous MLR.
groups. However, the yield of DC (mean ± SD) from IDDM relatives was markedly reduced (7.8 ± 3.2 × 10^7/10^7 PBMC) compared with paired controls (17 ± 5.1 × 10^6, p = 0.0002; Fig. 1). Similarly, the yield of DC from each IDDM patient was reduced compared with control (Table II).

Altered phenotype of DC from IDDM relatives

Up-regulation of the MHC class II molecule, HLA-DR, and the MHC class I-like molecule, CD1a, and down-regulation of the monocyte marker, CD14, characterizes the adherent monocyte-derived DC (14–17). The DR^{high}/CD14^{low} phenotype was displayed by 90 ± 8.8% (mean ± SD) of DC generated from IDDM relatives and 95 ± 3.6% from controls (Fig. 2A). However, compared with controls, a significantly lower proportion of cells from IDDM relatives expressed CD1a (72 ± 20 vs 90 ± 11%, p = 0.01; Fig. 2B) and the costimulator ligands CD80 or B7-1 (36 ± 25 vs 71 ± 11%, p = 0.001; Fig. 2C) and CD86 or B7-2 (34 ± 24 vs 62 ± 22%, p = 0.02; Fig. 2D). The proportions of cells that expressed CD54 or ICAM-1 (81 ± 23 vs 92 ± 10%) and CD58 or LFA-3 (76 ± 28 vs 89 ± 13%) were not significantly different. The only IDDM relatives in whom the proportion of B7-1-positive cells was not significantly decreased compared with HLA class II-matched controls were the four who were DR4,4;DQ7 or 8 homozygous. Otherwise, there were no apparent HLA associations.

The expression levels of both B7-1 and B7-2 per cell, measured as MFI units (mean ± SD), were significantly lower in IDDM relatives than in controls (B7-1, 390 ± 195 vs 537 ± 103, p = 0.02; B7-2, 482 ± 148 vs 586 ± 157, p = 0.01). However, the levels of HLA-DR (89.9 ± 8.8 vs 95.5 ± 3.6) and CD1a (654 ± 119 vs 689 ± 74) were no different. The differences in B7-1 and B7-2 expression can be clearly seen in representative examples (Fig. 3, A and B) of histograms overlaid from IDDM relatives and paired control subjects.

Impaired autologous MLR elicited by DC from IDDM relatives

Autologous CD4 T cells displayed a significantly lower proliferative response to DC from IDDM relatives than from controls at stimulator:responder ratios of 1:10 to 1:50. For example, at 1:10 (10^4 DC, 10^5 CD4 T cells), [3H]thymidine uptake (cpm) for IDDM relatives vs controls was 27,596 ± 14,691 vs 42,308 ± 10,893 (p = 0.04) (Fig. 4A). The response of allogeneic PBMC (from an HLA-DR 15,8;DQ2,7 individual) to DC from IDDM relatives compared with controls was decreased, but not significantly (Fig. 4B).

DC from IDDM relatives and patients with IDDM have similar defects

Monocyte-derived DC from three patients with recently diagnosed IDDM (Table II) displayed similar properties to DC from at-risk IDDM relatives vs controls was 27,596 ± 14,691 vs 42,308 ± 10,893 (p = 0.04) (Fig. 4B). The response of allogeneic PBMC (from an HLA-DR 15,8;DQ2,7 individual) to DC from IDDM relatives compared with controls was decreased, but not significantly (Fig. 4B).

DC from IDDM relatives and patients with IDDM have similar defects

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Discussion

This is the first demonstration of DC abnormalities in humans with preclinical autoimmune disease. At-risk IDDM relatives with underlying islet autoimmunity but with no metabolic dysfunction of diabetes had impaired yield, phenotype, and function of DC generated from peripheral blood adherent cells in the presence of GM-CSF and IL-4. Expression of B7-1 and B7-2 costimulator molecules was decreased on DC from IDDM relatives, together with the autologous MLR in response to these DC. Although impairment of CD4 T cell function in IDDM relatives was not directly excluded in this study, we think this is an unlikely explanation for the impaired autologous MLR. First, DC are unique in their ability to stimulate T cells in the autologous MLR (18), and their decrease in B7 expression would provide an explanation for the impaired autologous MLR. Second, a significant defect in T cell function was not revealed in the allogeneic MLR. The allogeneic stimulus is strong, and the response might be less affected by changes in DC phenotype such as a decrease in the expression of B7. Third, we (19) and others (20, 21) have found that T cell responses to both islet and nonislet Ags in preclinical at-risk relatives and patients with recently diagnosed IDDM are increased, not decreased.

Because MHC molecules have a direct role in Ag presentation, as well as other possible indirect effects on DC function, we carefully matched relatives with MHC class II identical controls. We found no difference in the expression of HLA-DR between relatives and controls. Therefore, MHC class II molecules can probably be eliminated as a variable that accounts for the depressed

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**Table III. Mouse mAbs used for phenotypic analysis of DC**

<table>
<thead>
<tr>
<th>CD Classification</th>
<th>mAb (Isotype)</th>
<th>Source*</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD1a</td>
<td>OKT6 (IgG1)</td>
<td>ATCC</td>
</tr>
<tr>
<td>CD14</td>
<td>M6P9 (IgG2b)</td>
<td>BD</td>
</tr>
<tr>
<td>CD54</td>
<td>84H10 (IgG1)</td>
<td>Dr. Andrew Boyd</td>
</tr>
<tr>
<td>CD58</td>
<td>TS29/1.4.3</td>
<td>ATCC</td>
</tr>
<tr>
<td>CD80</td>
<td>MAB104 (IgG1)</td>
<td>Immunotech</td>
</tr>
<tr>
<td>CD86</td>
<td>33401A (IgG1)</td>
<td>PharMingen</td>
</tr>
<tr>
<td>HLA DR</td>
<td>L243 (IgG2a)</td>
<td>ATCC or BD</td>
</tr>
</tbody>
</table>

* Source: BD, Becton Dickinson; Dr. Andrew Boyd (Walter and Eliza Hall Institute of Medical Research, Melbourne, Australia); Immunotech (Marseille, France); PharMingen (San Diego, CA); ATCC, American Type Culture Collection (Manassas, VA).
autologous MLR in relatives. DC phenotype and function varied considerably between individuals, but heterogeneity of phenotype and function within groups did not appear to be related to HLA class II phenotype. However, all subjects shared at least two HLA risk alleles for IDDM, and the number of alleles or haplotypes was insufficient for statistical analysis.

DC generated from patients with recently diagnosed IDDM displayed similar changes to those from at-risk relatives, consistent with the view that these changes are associated with the underlying autoimmune disorder that leads to β cell destruction. The question arises whether the changes in DC are the cause or effect of islet autoimmunity. HLA DR and DQ matching at least suggests that impaired DC function is not due to genetic susceptibility at MHC class II loci, but whether impaired DC phenotype and function is a genetic or acquired trait could be answered only by studying identical twins or HLA identical sibling pairs that are discordant for islet autoimmunity. Nevertheless, impairment of DC function to present self-peptides on MHC class II molecules, reflected in the autologous MLR, may contribute to the development of autoimmune disease. In the first place, DC present self-peptides to nascent T cells in the thymus and mediate the process of negative selection that deletes high affinity, self-reactive T cells (6). If adult peripheral blood adherent cell-derived DC were to functionally mirror thymic DC, then negative selection of high affinity autoreactive T cells might be impaired and predispose to autoimmune disease. The Ag specificity of this process would be dictated by the MHC repertoire and not necessarily be restricted to IDDM autoantigens. Controlled studies should be undertaken to investigate whether DC phenotype and function are also impaired in humans at risk for other autoimmune diseases, although apart from IDDM, identification of individuals with preclinical disease remains problematic.

FIGURE 2. Altered phenotype of DC in IDDM relatives. DC were phenotyped by surface labeling with a panel of mouse mAbs (Table III) and analyzed by flow cytometry. A live gate was set by forward and side scatter and propidium iodide staining. Five to ten thousand cells with high forward scatter and side scatter were counted. Dotted lines join at-risk IDDM relatives and age-, sex-, and HLA-matched control subjects. Short horizontal lines indicate mean values.
In addition to mediating deletion of autoreactive T cells, DC may also elicit tolerance in the periphery by activating autoregulatory T cells. In the autologous MLR, CD4 T cells that proliferate in response to self-MHC class II-peptide complexes on DC (22, 23) have been shown to have several effector functions, including immunosuppression in vitro (23–25). Furthermore, it has been known for many years that the autologous MLR is depressed in autoimmune disease-susceptible mice (26), including nonobese diabetic (NOD) mice susceptible to IDDM (27, 28), and in humans (29, 30) with IDDM. These observations are consistent with the idea that impairment of DC function in autoimmune disease leads to deficient generation of regulatory T cells. Because autoantigen-specific T cells can be detected in healthy individuals, autoimmune disease can be viewed ultimately as a failure of peripheral regulatory mechanisms. The NOD mouse is characterized not only by a low autologous MLR (27, 28) but by decreased sensitivity of myeloid progenitor cells to GM-CSF (31) and by a defect in bone marrow-derived APC function (32). Clare-Salzler et al. (7) found that when young NOD mice were given a single s.c. injection of DC purified from pancreatic but not other lymph nodes of adult NOD mice, their incidence of diabetes was significantly reduced. Pooled lymph node cells from the recipient mice were then shown to suppress the adoptive transfer of diabetes by splenic T cells from diabetic mice. This implies that the transfer of pancreatic lymph node DC, presumably loaded with islet Ag(s), induced regulatory cells. Recent studies have identified both CD4 (33, 34) and CD8 (35) regulatory T cells in NOD mice, generated by the mucosal administration of islet Ags (insulin, glutamic acid decarboxylase) that are associated with Th2 cytokine profiles and inhibit adoptive transfer of diabetes. CD1, an MHC class I-like molecule, has been implicated as a ligand for two different types of regulatory T cells, mucosal intraepithelial CD8 T cells in oral tolerance (36) and NK1.1 T cells that secrete the Th2 cytokine IL-4 (37). CD8 γδ T cells that suppress diabetes in the NOD mouse are induced by administration of aerosol insulin to the mucosa (35), and NK1.1 T cells are deficient in NOD mice (38). In IDDM relatives, the expression of CD1a per cell, in contrast to B7-1 and B7-2, was normal, but the yield of CD1a+ cells generated was decreased. Whether regulatory T cells are induced by Ag presentation on CD1 or other molecules that may be altered on DC, including mucosal DC, is a question that must be addressed.

At a molecular level, how could DC fail to efficiently generate regulatory T cells and thereby promote autoimmune disease? T cell responses through the T cell receptor are regulated by B7 molecules in a manner that is complex and still controversial. Impaired ligation of CD28 on T cells to B7-1 and B7-2 on DC may lead to suboptimal expression of T cell molecules required for T cell clonal expansion (IL-2R, IL-2, and CD40 ligand) and viability (Bcl-xL) (39). A variety of experimental models have shown that costimulation through CD28 is necessary for priming of Th2 cells, without which T cells default to the Th1 subset (40–44). Decreased expression of B7 molecules may therefore impair the generation of Th2 cells and shift the immune balance toward Th1, thereby promoting cell-mediated disease such as IDDM. In fact, progression of diabetes in the NOD mouse was accelerated in the presence of a CD28 deficiency (43). The situation is unlikely to be this simple, however. For instance, the differential interaction of B7-1 vs B7-2 is reported to influence the nature of the T cell response, with B7-2 promoting Th2 responses (8, 45). Furthermore, T cell activation is followed by up-regulation of CTLA-4, which also ligates to B7-1 and B7-2, but with significantly higher affinity than CD28 (46). Therefore, decreased expression of B7 molecules could favor interaction with CTLA-4 over CD28 on activated T cells. How signaling through CD28 and CTLA-4 is normally integrated and the effect of its perturbation on T cell function are not fully understood. In purified mouse T cells, CD28 and CTLA-4 deliver opposing positive and negative signals, respectively (47), although the claim that CTLA-4 is solely a negative regulator has been challenged (48). In any event, as well as decreased B7 signaling through CD28, the differential interaction of B7 with CTLA-4 might also impair generation of Th2 cells with regulatory properties.

In concluding, our findings in humans with islet autoimmunity would support the hypothesis that a defect in DC function, in the thymus to reduce editing of self-reactive T cells or in the periphery to impair induction of regulatory T cells, predisposes to IDDM.
**FIGURE 4.** Impaired Ag-presenting capacity of DC to autologous CD4 T cells in IDDM relatives. Autologous CD4 cells (1 × 10^6) positively selected with magnetic beads (A) or allogeneic PBMC (2 × 10^5) from one individual (B) were cultured in 96-well round-bottom microplates with 1 × 10^6 irradiated (3000 rad, ^131^I Cs source) stimulator DC. Proliferation was measured on day 5 by a 6-h pulse with [%H]thymidine. Dotted lines join at-risk IDDM relatives and age-, sex-, and HLA-matched control subjects. Short horizontal lines indicate mean values.

**References**


