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Inhibition of Autoimmune Diabetes in Nonobese Diabetic Mice by Transgenic Restoration of H2-E MHC Class II Expression: Additive, But Unequal, Involvement of Multiple APC Subtypes

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Transgenic restoration of normally absent H2-E MHC class II molecules on APC dominantly inhibits T cell-mediated autoimmune diabetes (IDDM) in nonobese diabetic (NOD) mice. We analyzed the minimal requirements for transgenic H2-E expression on APC subtypes (B lymphocytes vs macrophages/dendritic cells (DC)) to inhibit IDDM. This issue was addressed through the use of NOD stocks transgenically expressing high levels of H2-E and/or made genetically deficient in B lymphocytes in a series of genetic intercross and bone marrow/lymphocyte chimera experiments. Standard (H2-E\textsuperscript{null}) NOD B lymphocytes exert a pathogenic function(s) necessary for IDDM. However, IDDM was inhibited in mixed chimeras where H2-E was solely expressed on all B lymphocytes. Interestingly, this resistance was abrogated when even a minority of standard NOD H2-E\textsuperscript{null} B lymphocytes were also present. In contrast, in NOD chimeras where H2-E expression was solely limited to approximately half the macrophages/DC, an active immunoregulatory process was induced that inhibited IDDM. Introduction of a disrupted IL-4 gene into the NOD-H2-E transgenic stock demonstrated that induction of this Th2 cytokine does not represent the IDDM protective immunoregulatory process mediated by H2-E expression. In conclusion, high numbers of multiple subtypes of APC must express H2-E MHC class II molecules to additively inhibit IDDM in NOD mice. This raises a high threshold for success in future intervention protocols designed to inhibit IDDM by introduction of putatively protective MHC molecules into hemopoietic precursors of APC. The Journal of Immunology, 2001, 167: 2404–2410.

Type 1 diabetes (insulin-dependent diabetes mellitus, IDDM)\textsuperscript{3} in both humans and nonobese diabetic (NOD) mice results from autoimmune destruction of insulin-producing pancreatic \( \beta \) cells mediated by both CD4 and CD8 T cell responses (reviewed in Refs. 1 and 2). Multiple susceptibility (\textit{Idd}) genes contribute to IDDM development (reviewed in Refs. 3 and 4). However, while polygenically controlled, particular MHC haplotypes provide the primary genetic component of IDDM susceptibility in both humans and NOD mice (reviewed in Refs. 4 and 5). Within the MHC, specific combinations of HLA-DQ and -DR class II alleles provide a large component of IDDM susceptibility in humans by mediating \( \beta \) cell autoreactive CD4 T cell responses (5). Similarly, IDDM development in NOD mice requires that the rare H2-A\textsuperscript{g7} MHC class II gene product (homologue of human DQ8) be homozygously expressed. This was demonstrated by the fact that transgenes encoding H2-A\textsuperscript{g7} variants from diabetes-resistant MHC haplotypes confer dominant IDDM resistance to NOD mice (6–10). However, NOD mice also normally fail to express an H2-E MHC class II variant (homologue of human DR) due to a deletion in the first exon of the gene encoding its \( \alpha \)-chain subunit (11). The lack of H2-E expression also contributes to IDDM susceptibility in NOD mice, because reversal of this defect with \( \varepsilon \) transgenes inhibits the development of disease (7, 12, 13). Certain human MHC class II gene products, such as DQ6, also appear to confer dominant IDDM resistance (5). Because DR products are always expressed on human APC, it is unclear whether specific alleles in linkage disequilibrium with diabetogenic DQ alleles actually contribute to increased disease risk, are neutral (confering no protection), or confer different degrees of protection (14).

MHC class II expression is largely restricted to hemopoietically derived APC, which include B lymphocytes, macrophages, and DC (reviewed in Ref. 15). Hence, it is not surprising that APC defects controlled by the unusual class II variants of the H2\textsuperscript{g7} MHC haplotype, but also partially dependent on contributions from other \textit{Idd} genes, are largely responsible for IDDM development in NOD mice (reviewed in Ref. 2). These defects entail a reduced ability of NOD APC to mediate tolerogenic functions that would normally block the development of autoreactive T cells. APC are also essential to the subsequent functional activation of the pancreatic \( \beta \) cell autoreactive CD4 T cells that are generated as a result of these tolerance induction defects in NOD mice. B lymphocytes have been shown to be the most important subpopulation of APC in NOD mice for activating the effector activity of \( \beta \) cell autoreactive CD4 T cells (16–19). Collectively, these previous findings indicate that APC could be an ideal target for gene therapy protocols that may inhibit IDDM development. The transgenic studies described above indicate that an effective IDDM prevention protocol might be one that causes APC to express a dominantly protective MHC class II gene product. However, a prerequisite to the success of such protocols is to determine what

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\textsuperscript{3} Abbreviations used in this paper: IDDM, type 1 diabetes, insulin-dependent diabetes mellitus; NOD, nonobese diabetic; MIS, mean insulitis score.
particular subtype(s) and proportions of APC must express a particular MHC class II variant to inhibit IDDM development. Furthermore, the ability to use APC expressing potentially protective MHC class II variants to block IDDM development might also be enhanced by determining their disease inhibitory mechanisms.

In the current study we evaluated what subtypes and proportions of APC must express transgenically restored H2-E MHC class II molecules to inhibit IDDM development in NOD mice and tested a previously proposed mechanism of protection. The issue of what APC subtypes exert H2-E-mediated IDDM protective effects was addressed through the use of Ea transgenic NOD mice that were made deficient in B lymphocytes by introduction of a functionally inactivated Igα gene (designated Igαmnull). We had previously reported that a possible mechanism by which IDDM development is inhibited by transgenic H2-E expression in NOD mice might entail an alteration in the pattern of cytokines produced by β cell autoreactive CD4 T cells from a Th1 (IFN-γ) to a Th2 (IL-4, IL-10) profile (13). This possibility was tested by the introduction of a functionally inactivated IL-4 gene into our Ea transgenic NOD stock.

Materials and Methods

Mice

NOD/Lt mice are maintained in a specific pathogen-free research colony at The Jackson Laboratory (Bar Harbor, ME). Currently, IDDM develops in 90% of female and 65% of male NOD/Lt mice by 1 yr of age. A stock of NOD mice made completely IDDM resistant by direct introduction of an H2-Eαm transgene (formal designation NOD/Lt-TgN (H2-Eαm)lnN5; here designated NOD-H2-E for simplicity) has been previously described (13). Derivation of an N10 backcross stock of NOD mice made IDDM resistant through the elimination of B lymphocytes by a functionally disrupted Igα allele (official designation NOD.Igh6 tm1Cgn; here designated NOD.Igαmnull), and its use to homogenize for linkage markers delineating all previously identified Idd loci of NOD origin has also been previously described (20). These latter two stocks served as progenitors for a line of B lymphocyte-deficient NOD-H2-E mice (designated NOD-H2-E.Igαmnull), NOD-H2-E and NOD.Igαmnull mice were intercrossed. The resulting F1 hybrids were backcrossed to NOD.Igαmnull mice to produce progeny homozygous for the Igαmnull mutation and heterozygous for the H2-E transgene. Homozygous Igαmnull segregants were identified by the absence of B lymphocytes among PBL using the previously described flow cytometric technique (20). Heterozygous H2-E transgene carriers were identified by the previously described Southern blot analysis of tail snap DNA (13). We had also previously produced (21, 22) an N9 backcross stock of NOD mice homozygous for both a functionally disrupted IL-4 gene and linkage markers delineating all known Idd loci of NOD origin (formal designation NOD.Il4mnull; here designated NOD.Ildmnull). The availability of this stock allowed for the generation of IL-4-deficient NOD-H2-E mice (designated NOD-H2-E.Il4mnull). F1 hybrids between NOD.Ildmnull and NOD-H2-E mice were backcrossed to the NOD.Ildmnull stock. Segregants homozygous for the Ildmnull allele were detected by the previously described PCR assay (21), while carriers of the H2-E transgene were identified by flow cytometry for positive staining of PBL with the FITC-conjugated monoclonal Ab 1b4-4S. All mice were allowed free access to food (National Institutes of Health 31A/6% fat diet; Ralston Purina, Richmond, IN) and acidified drinking water.

Assessment of diabetes development

IDDM development in the indicated mice was assessed by weekly monitoring of glycosuric values with Ames Diastrix (supplied by Bayer, Diagnostic Division, Elkhart, IN). Values of ≥3 were considered indicative of IDDM onset.

Generation of mixed bone marrow/B lymphocyte chimera

In some experiments, 4- to 6-wk-old female NOD mice were lethally irradiated (1200 rad from a 137Cs source) and reconstituted as previously described (23) with 5 × 108 T cell-depleted syngeneic bone marrow cells admixed with various numbers of T cell-depleted marrow cells from the indicated partner donor strains. Other experiments used female NOD.Igαmnull mice reconstituted at 4–6 wk of age with 5 × 108 T cell-depleted syngeneic marrow cells admixed with the indicated numbers of purified B lymphocytes from standard NOD and/or NOD-H2-E donors. Splenic B lymphocytes were purified using the previously described magnetic bead system (16). The purity of B lymphocytes isolated by this technique routinely exceeded 93%. Another experimental group consisted of NOD.Igαmnull females reconstituted at 4–6 wk of age with 5 × 109 T cell-depleted NOD-H2-E.Igαmnull marrow cells combined with 3 × 106 purified NOD B lymphocytes. Positive controls for all experimental groups consisted of NOD or NOD.Igαmnull females reconstituted at 4–6 wk of age with 5 × 109 T cell-depleted NOD bone marrow cells. Control and experimental chimeras were monitored for IDDM development as described above for 21-wk postreconstitution. Upon developing IDDM or reaching the 21-wk postreconstitution end point, types and proportions of H2-E expressing APC in the various chimeras were determined by FACS analysis as described below. In addition, pancreases from chimeras remaining free of overt IDDM for the 21-wk postreconstitution period were histologically examined for insulitis development as described below.

Quantification of H2-E-expressing APC

Splenic leukocytes from the indicated chimeras were assessed by multicolor FACS analysis (FACScan; BD Biosciences, San Jose, CA) for proportions and types of APC expressing H2-E at the time of IDDM development or at 21 wk postreconstitution. Previous analyses found that maximal chimerization levels of all pertinent cell types were achieved at 4–6 wk postreconstitution and did not vary after this time. All analyses used the CellQuest 3.0 data reduction system (BD Biosciences). Total numbers of APC were defined by the presence of MHC class II expression. All APC in each type of chimera used in these studies expressed the NOD H2-A1, H2-D3, and H2-E MHC class II gene products, which was detected with mAb AMS32.1 conjugated to a red fluorescent PE tag. The proportion of total APC that also expressed the H2-E MHC class II transgene product was detected by staining splenic leukocytes with mAb 14-4-4S conjugated to a green fluorescent FITC tag. Separate aliquots of splenic leukocytes were assessed for containing with the FITC-conjugated 14-4-4S Ab and the PE-conjugated RA3-6B2 Ab specific for the B220 cell surface marker to determine the proportion of B lymphocytes that expressed H2-E. The proportions of macrophages/DC that expressed H2-E were determined by staining of splenic leukocytes with the FITC-conjugated 14-4-4S Ab and the PE-conjugated M1/70 Ab specific for the Mac-1 cell surface marker. Data are presented as the mean proportion ± SEM of total APC, B lymphocytes, or macrophages/DC that expressed H2-E in the indicated experimental group.

Histological analyses of insulitis development

The indicated chimeric mice remaining free of overt IDDM for 21-wk postreconstitution were assessed for insulitis development. Pancreases were fixed in Bouin’s solution and sectioned at three nonoverlapping levels. Granulated β cells were stained with aldehyde fuchsin, and leukocytes were stained with a H&E counterstain. Islets (at least 20/mouse) were individually scored as follows: 0, no lesions; 1, peri-insular leukocytic infiltration; 2, 25%/islet destruction; 3, 25–75% islet destruction; 4, complete islet destruction. An insulitis score for each mouse was obtained by dividing the total score for each pancreas by the number of islets examined. Data are presented as the mean insulitis score (MIS) ± SEM for the indicated experimental group.

Results

H2-E-expressing B lymphocytes inefficiently block IDDM development in NOD mice

Previous transgenic studies in NOD mice indicated that a protocol that induces the expression of a dominantly protective MHC class II variant in APC might ultimately prove to be clinically useful in blocking IDDM development in otherwise susceptible individuals. However, a necessary prerequisite for such a protocol is to determine what subtype(s) of APC must express a particular protective MHC class II variant to most efficiently inhibit IDDM. We had previously found that a major component of IDDM resistance elicited by transgenic restoration of H2-E MHC class II expression in NOD mice entailed the induction of an extrathymic immunoregulatory process (13). B lymphocytes are rare in the thymus, but represent the most prevalent subtype of APC in the periphery. Thus, we hypothesized that the peripheral IDDM protective immunoregulatory process engendered by transgenic H2-E expression in NOD mice is mediated by B lymphocytes. We tested this...
possibility through use of our previously described chimeric system (16) that allowed NOD T cells to mature and function in an environment where transgenic H2-E expression is solely restricted to a selected proportion of B lymphocytes. These chimeras were generated by reconstituting lethally irradiated NOD.IgMnull recipients with syngeneic marrow admixed with various proportions of purified B lymphocytes from NOD and/or NOD-H2-E donors. This particular chimeric approach must be used because unmanipulated NOD.IgMnull mice are not tolerant of even standard NOD B lymphocytes and hence reject them upon direct infusion (16).

Table I summarizes the mean proportion of H2-E-positive and -negative B lymphocytes that repopulated spleens of each type of chimera analyzed. Also depicted is the proportion of total APC (defined as all MHC class II-positive cells) in each type of chimera that coexpressed H2-E. Fig. 1 shows IDDM development as a function of the percentage of H2-E-expressing splenic B lymphocytes that developed in each group of mixed chimeras. As expected from a previous study (16), IDDM developed over a 21-wk follow-up period in most NOD.IgMnull females (65.0%, 15 of 23) reconstituted with syngeneic marrow and standard H2-E-negative NOD B lymphocytes (Fig. 1). In contrast, over the same period of time IDDM developed in 11.1% (one of nine) of NOD.IgMnull females reconstituted with syngeneic marrow and only NOD-H2-E B lymphocytes. NOD.IgMnull mice that remained free of overt IDDM following reconstitution with syngeneic marrow admixed only with NOD-H2-E B lymphocytes were also characterized by moderate levels of insulitis (MIS, 1.90 ± 0.49; n = 8). The above chimeras expressed H2-E solely on all B lymphocytes (80.8% of all APC). To address the minimal proportions of H2-E-expressing B lymphocytes required to elicit IDDM resistance, we also analyzed chimeras in which H2-E was expressed on 0–50%, 50–75%, or >75% of B lymphocytes (Table I). This corresponded to mean proportions of 13.6, 34.9, and 62.8% of total APC expressing H2-E. Unlike the chimeras in which all B lymphocytes expressed H2-E, none of the chimeras characterized by these lower levels of H2-E-expressing B lymphocytes was significantly protected from IDDM (Fig. 1). Hence, NOD B lymphocytes transgenically expressing H2-E-MHC class II molecules can inhibit IDDM development, but cannot do so when H-2-negative B lymphocytes are also present. An important implication of these results is that transgenic H2-E expression on all B lymphocytes is unlikely to inhibit IDDM development in NOD mice by increasing the ability of these APC to mediate a protective peripheral immunoregulatory mechanism. Rather, when all NOD B lymphocytes express transgenic H2-E molecules, they most likely lose some pathogenic function(s) normally necessary to IDDM development.

Transgenic H2-E expression on all macrophages/DC efficiently inhibits IDDM development in NOD mice

The preceding results indicated that H2-E-expressing B lymphocytes cannot efficiently inhibit IDDM development in NOD mice. This suggested that a significant level of protection might be achieved when H2-E was exclusively expressed on macrophages/DC. However, if H2-E must be simultaneously expressed on multiple types of APC to confer significant protection from IDDM, then restricting expression of such class II molecules solely to macrophages/DC would also prove to be an ineffective means of

![Graph](http://www.jimmunol.org/)

**FIGURE 1.** Minimum requirement for IDDM protection mediated by H2-E-expressing B lymphocytes. NOD.IgMnull female mice were lethally irradiated at 4–6 wk of age and reconstituted with 5 × 10⁶ syngeneic bone marrow cells admixed with 3 × 10⁶ purified B lymphocytes from NOD (++; n = 23) or NOD-H2-E (●; n = 9) donors. Other NOD.IgMnull female mice were reconstituted with syngeneic marrow together with three different mixtures of NOD and NOD-H2-E B lymphocytes (group 1, 5 × 10⁶ of each; group 2, 2 × 10⁶; 6 × 10⁶; group 3, 1 × 10⁶; 10 × 10⁶). This respectively produced chimeras in which 0–25% (○; n = 21), 25–50% (▲; n = 14), or >50% (●; n = 6) of the B lymphocytes expressed H2-E. All chimeras were monitored for IDDM development for 21-wk postreconstitution. *IDDM development significantly less (p < 0.05, Kaplan-Meier life table analysis) than in NOD.IgMnull controls reconstituted with syngeneic marrow and NOD B lymphocytes.

<table>
<thead>
<tr>
<th>Table I. Repopulation by H2-E expressing B lymphocytes in NOD.IgMnull chimeric recipientsa</th>
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<tr>
<td>Cell Types Used To Reconstitute NOD.IgMnull Recipients</td>
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<tr>
<td>NOD.IgMnull BM + NOD B cells (n = 23)</td>
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<tr>
<td>NOD.IgMnull BM + NOD-H2-E B cells (n = 9)</td>
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<tr>
<td>NOD.IgMnull BM + mixed NOD and NOD-H2-E B cells (group 1: 50–75% H2-E B cells; n = 6)</td>
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<tr>
<td>NOD.IgMnull BM + mixed NOD and NOD-H2-E B cells (group 2: 25–50% H2-E B cells; n = 14)</td>
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<tr>
<td>NOD.IgMnull BM + mixed NOD and NOD-H2-E B cells (group 3: &lt;25% H2-E B cells; n = 21)</td>
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a Female NOD.IgMnull mice were lethally irradiated at 4–6 wk of age and reconstituted with the indicated mixtures of bone marrow and B lymphocytes (total cell numbers used for reconstitution provided in Fig. 1). Proportions of splenic H2-E-expressing B lymphocytes and total APC were assessed either at the onset of overt IDDM or at 21 wk postreconstitution.
b Represents proportion of B lymphocytes (identified by staining with the B220-specific Ab RA3-6B2) expressing H2-E (detected by staining with the 14-4-4S Ab).
c Represents proportion of total MHC class II-positive cells (identified by staining with AMS32.1 Ab) expressing H2-E (detected by staining with the 14-4-4S Ab).
inhibiting disease development. To distinguish between these possibilities, we determined the extent of IDDM development in chimeric NOD mice that expressed H2-E on all macrophages/DC, but not B lymphocytes. These were generated by reconstituting NOD.\textit{Ig}^{\textit{mu}}\textit{null} female recipients with NOD-H2-E.\textit{Ig}^{\textit{mu}}\textit{null} bone marrow admixed with purified standard NOD B lymphocytes. Controls consisted of NOD.\textit{Ig}^{\textit{mu}}\textit{null} female recipients reconstituted with syngeneic marrow plus NOD B lymphocytes. As expected, over a 21-wk follow-up period, IDDM developed in most (71.4%, five of seven) of these control chimeras (Fig. 2). In contrast, over the same period of time, IDDM developed in a significantly lower proportion (16.7%, two of 12) of the NOD.\textit{Ig}^{\textit{mu}}\textit{null} females reconstituted with NOD-H2-E.\textit{Ig}^{\textit{mu}}\textit{null} bone marrow plus NOD B lymphocytes. Furthermore, the NOD.\textit{Ig}^{\textit{mu}}\textit{null} females that remained free of overt IDDM following reconstitution with NOD-H2-E.\textit{Ig}^{\textit{mu}}\textit{null} bone marrow plus NOD B lymphocytes were characterized by significantly lower levels of insulitis (MIS, 1.33 ± 0.36; \textit{n} = 10) than the few control chimeras that remained free of overt disease (MIS, 3.05 ± 0.94; \textit{n} = 2). Hence, IDDM was inhibited in NOD mice under conditions where transgenic H2-E molecules are expressed on all macrophages/DC, but not any B lymphocytes.

Minimum requirement for H2-E-expressing macrophages/DC to inhibit IDDM

We hypothesized that if H2-E-expressing macrophages/DC inhibited IDDM by inducing an active immunoregulatory mechanism, then they would only need to comprise some minimal threshold proportion of all APC to manifest their protective effect. To initially test this possibility, we compared the extent of IDDM development in standard NOD female mice reconstituted with syngeneic marrow alone or admixed with an equal number of marrow cells from NOD-H2-E donors. As expected, about 50% of all APC in NOD females reconstituted with a 1/1 mixture of NOD and NOD-H2-E marrow expressed H2-E (Table II). It is important to note that unlike the mice depicted in Fig. 1 in which the only H2-E-expressing APC were B lymphocytes, in this experiment the 50% of H2-E-expressing APC also included macrophages/DC. Fig. 3 shows that over the 21-wk observation period, IDDM developed in a significantly smaller proportion of the chimeras reconstituted with a 1/1 mixture of NOD and NOD-H2-E marrow (33.3%, 8 of 24), than NOD marrow alone (91.7%, 11 of 12). Insulitis levels in chimeras that did not develop overt IDDM following reconstitution with a 1/1 mixture of NOD and NOD-H2-E marrow (MIS, 1.43 ± 0.3; \textit{n} = 14) were also much lower than that in the single control chimera that failed to develop overt disease (IS, 3.9)

As shown in Fig. 1, H2-E expression solely on ~50% of B lymphocytes did not inhibit IDDM development. Hence, the ability of H2-E to also be expressed on macrophages/DC most likely accounted for disease protection in the chimeras depicted in Fig. 3 that were reconstituted with a 1/1 mixture of NOD and NOD-H2-E marrow. To test this hypothesis, we assessed IDDM development in chimeras where transgenic H2-E molecules were only expressed on a fraction of macrophages/DC, but not on B lymphocytes. This was done by reconstituting NOD female recipients with syngeneic marrow admixed with an equal number of marrow cells from NOD-H2-E.\textit{Ig}^{\textit{mu}}\textit{null} donors. Such an approach generated chimeras that could be stratified on the basis of H2-E-expressing macrophages/DC comprising either more or less than 5% of the total APC (Table II). As shown in Fig. 3, the only chimeras that developed IDDM at a rate significantly less (42.9%, three of seven) than controls reconstituted with NOD marrow alone (91.7%), were those in which H2-E-expressing macrophages/DC comprised >5% of the total APC. In these IDDM-resistant chimeras, the mean proportion of H2-E-expressing macrophages/DC among total MHC class II-positive APC was 7.3 ± 0.5% (Table II). This value represents a little over half of the total macrophages/DC. Such a finding, combined with the fact that chimeras reconstituted with a 1/1 mixture of NOD and NOD-H2-E marrow are also IDDM resistant, indicates that H2-E-mediated disease protection requires that these MHC class II molecules be expressed on about 50% of macrophages/DC, which represents about 5% of all APC.

Production of the Th2 cytokine IL-4 is not required for the induction of IDDM resistance in NOD-H2-E mice

A number of Ag-specific and nonspecific immunostimulatory protocols that inhibit IDDM development in NOD mice are associated with a shift in the cytokines produced by islet-infiltrating T cells from a Th1 (primarily IFN-\gamma) to a Th2 (primarily IL-4) profile (reviewed in Refs. 1, 24, and 25). We had previously found that following priming and restimulation with the 65-kDa variant of the candidate \( \beta \) cell autoantigen glutamic acid decarboxylase, T cells from the NOD-H2-E transgenic stock produced significantly higher levels of the Th2 cytokine IL-4 than similar T cells from standard NOD mice (13). Thus, we hypothesized that H2-E-mediated IDDM inhibition might result from an increased ability of \( \beta \) cell autoreactive T cells to produce the potentially protective Th2 cytokine IL-4. We subsequently developed a fully IDDM-susceptible NOD congenic stock genetically deficient in IL-4 (21). The availability of this stock allowed us to produce through intercrossing, NOD-H2-E transgenic mice with either an intact or a genetically disrupted IL-4 gene. As expected, by 30 wk of age, IDDM had developed in a high proportion (73.7%, 14 of 19) of female IL-4 intact, H2-E-negative, NOD control segregants (Fig. 4). Also, as expected based on previously published results (22), over the same period of time IDDM developed in a high proportion (68.8%, 11 of 16) of IL-4-deficient H2-E-negative congenic females. However, transgenic H2-E expression remained capable of completely suppressing IDDM development in IL-4-deficient females (0%, 0 of 14). Hence, the inhibition of IDDM development in NOD mice by transgenic restoration of H2-E MHC class II expression is not dependent upon the induction of IL-4 production by \( \beta \) cell autoreactive T cells.
Number of NOD-H2-E marrow cells (\(n = 12\)) or admixed with an equal number of NOD-H2-E marrow cells (\(n = 24\)). Other NOD female mice were lethally irradiated at 4–6 wk of age and reconstituted with 5 × 10^6 syngeneic bone marrow cells alone (\(\bullet\), \(n = 12\)) or admixed with an equal number of NOD-H2-E marrow cells admixed with the same number of NOD-H2-E-Ignull marrow cells. This latter approach generated chimeras in which H2-E expression was restricted solely to macrophages/DC and which could be stratified on the basis of such cells comprising either greater than (\(\Delta\), \(n = 7\)) or less than (\(\bullet\), \(n = 10\)) 5% of the total MHC class II positive APC.

**Discussion**

Previous studies in NOD mice have suggested that developing a hemopoietic stem cell-based protocol to induce APC to express a dominantly protective MHC class II allele might ultimately provide a way to inhibit the development of autoimmune IDDM in otherwise susceptible individuals (26). A prerequisite for developing such protocols is determining what types and proportions of marrow-derived APC must express a particular dominantly protective MHC class II variant to inhibit IDDM. In the present study we demonstrate through chimeric analyses, that strong, but incomplete, IDDM resistance was obtained when H2-E was expressed solely on all B lymphocytes. However, this IDDM protective effect was lost when even a minority population of H2-E-negative B lymphocytes were also present. Strong, but incomplete, IDDM protection was also observed when H2-E was solely expressed on macrophages/DC. In this latter case IDDM protection was still observed as long as H2-E was expressed on at least half of all macrophages/DC, which comprised ~5% of the total MHC class II-positive APC. Significantly, the extent of IDDM protection observed when H2-E was solely expressed on approximately half of the macrophages/DC was the same as that obtained when H2-E was expressed on half of all APC. This indicates that transgenic restoration of H2-E expression on half of the macrophages/DC and B lymphocytes does not result in a synergistic enhancement of IDDM resistance. Collectively, these results demonstrate that transgenic restoration of H2-E expression on B lymphocytes or macrophages/DC can inhibit IDDM development in NOD mice, but there are differences in the proportions of these APC subtypes that must express H2-E to elicit protective effects. Furthermore, based on their ability to inhibit disease at quantitatively lower levels than B lymphocytes, macrophages/DC appear to represent the most efficient subtype of APC for mediating H2-E-induced IDDM protection.

There are several mechanistic implications of the finding that different percentages of B lymphocytes vs macrophages/DC must express H2-E to exert IDDM protective effects in NOD mice. The first of these is that complete IDDM resistance in the NOD-H2-E stock (all APC express H2-E molecules) should additively result from the partially protective effects that are engendered when H2-E is expressed on all B lymphocytes and at least 50% of macrophages/DC. Furthermore, the finding that the majority, if not all, B lymphocytes must express H2-E to inhibit IDDM development indicates such protection does not result from this APC subset acquiring an ability to actively mediate an immunoregulatory mechanism(s). Rather, the expression of H2-E on most or all B lymphocytes most likely results in their loss of some pathogenic function(s) normally necessary to IDDM development. Such lost pathogenic functions could include those that allow B lymphocytes

**FIGURE 3.** H2-E-expressing macrophages/DC inhibit IDDM in NOD chimeric mice when comprising about 5% of total APC. NOD female mice were lethally irradiated at 4–6 wk of age and reconstituted with 5 × 10^6 syngeneic bone marrow cells alone (\(\bullet\), \(n = 12\)) or admixed with an equal number of NOD-H2-E marrow cells (\(\Box\), \(n = 24\)). Other NOD females were lethally irradiated at 4–6 wk of age and reconstituted with 5 × 10^6 syngeneic marrow cells admixed with an equal number of NOD-H2-E-Ignull marrow cells. This latter approach generated chimeras in which H2-E expression was restricted solely to macrophages/DC and which could be stratified on the basis of such cells comprising either greater than (\(\Delta\), \(n = 7\)) or less than (\(\bullet\), \(n = 10\)) 5% of the total MHC class II positive APC. All chimeras were monitored for IDDM development for 21 wk post-reconstitution.

**FIGURE 4.** Transgenic H2-E expression does not elicit IDDM resistance by increasing T cell production of the Th2 cytokine IL-4. IDDM development was assessed through 30 wk of age in female segregants generated from an intercross of NOD-H2-E and NOD IL4null mice that were IL-4 intact and H2-E negative (\(\bullet\), \(n = 19\)), deficient in both IL-4 and H2-E (\(\Delta\), \(n = 16\)), or IL-4 deficient and H2-E positive (\(\Box\), \(n = 14\)).
to serve as a preferential subpopulation of APC for activating autoreactive CD4 T cell responses against certain β cell autoantigens (16, 17). In contrast, the finding that H2-E only needs to be expressed on a portion of macrophages/DC to inhibit IDDM development in NOD mice suggests that such protection results from these APC acquiring the ability to activate a normally absent immunoregulatory function(s).

The question remains as to what sort of active IDDM protective immunoregulatory activities are gained by NOD macrophages/DC that transgenically express H2-E molecules. Macrophages/DC represent the most prevalent type of APC in the thymus. Hence, such cells are likely to mediate the previously reported intrathymic deletion of a highly diabetogenic CD4 T cell clonotype in H2-E-expressing NOD mice (27). However, H2-E expression does not result in the clonal deletion of a broad range of diabetogenic CD4 T cells. This was demonstrated by the finding that purified T cells from NOD-H2-E mice (liberated from the influence of H2-E-expressing APC) could adoptively transfer IDDM to lymphocyte-deficient NOD-scid recipients (13). These previous results also indicate that NOD diabetogenic T cells remain functionally suppressed, but are not anergized, as long as they remain in the presence of H2-E-expressing APC. Our current findings indicate that macrophages/DC represent the subtype of H2-E-expressing APC that maintain the peripheral functional suppression of diabetogenic NOD T cells.

We had previously obtained evidence that H2-E-expressing APC might functionally suppress β cell autoreactive CD4 T cells in NOD mice by enhancing their production of the Th2 cytokine IL-4, which has been proposed to dampen the pathogenic potential of such effectors (1, 13, 24, 25). This hypothesis was rejected in the current study, because transgenic restoration of H2-E expression remaining capable of completely inhibiting IDDM in NOD mice made genetically deficient in IL-4. It has also been proposed that through a mechanism termed determinant capture, transgenic H2-E molecules can inhibit IDDM development in NOD mice by binding pancreatic β cell peptides that are normally presented to autoreactive CD4 T cells by H2-A^87^ class II molecules, hence preventing the activation of such effectors (28). Partially arguing against the determinant capture mechanism is a report that none of 80 peptides derived from the candidate β cell autoantigen 65-kDa heat shock protein could strongly bind to both H2-A^87^ and transgenic H2-E MHC class II molecules (29). We had also previously found that transgenic H2-E expression might inhibit IDDM development in NOD mice by inducing a reciprocal decrease in the expression of endogenous H2-A^87^ MHC class II molecules on APC (13). However, it is difficult to envision that such a decrease in H2-A^87^ expression could increase the ability of APC to mediate an active immunoregulatory function such as that exerted by macrophages/DC from NOD-H2-E mice. In contrast, the triggering of a reciprocal decrease in H2-A^87^ expression on most all B lymphocytes in NOD-H2-E mice could account for this subset of APC losing a pathogenic function(s) normally necessary for IDDM development, such as their preferential ability to present certain β cell Ags to autoreactive CD4 T cells. Thus, while our current studies clearly demonstrate that IDDM resistance in NOD-H2-E mice is mediated through different effects by both B lymphocytes and macrophages/DC, the actual protective mechanisms exerted by these APC subsets remain an open question.

Our current results also illustrate an important obstacle that will have to be overcome before it will be possible to use APC made to express a single dominantly protective MHC class II gene product for blocking IDDM development in a clinical setting. This obstacle is that the clinical induction of IDDM resistance may require the expression of the appropriate class II variant in a very high proportion of a particular subtype of APC. In the case of H2-E in NOD mice, protection requires expression of this class II variant in at least half of all macrophages/DC and/or the vast majority of B lymphocytes. It would probably be difficult to maintain this level of protective APC by their direct infusion into IDDM-susceptible individuals. Instead, the most effective way of maintaining the necessary level of protective APC in such individuals would be to reconstitute them with essentially pure populations of hemopoietic stem cells that had been transfected with the appropriate MHC class II gene construct. Hemopoietic stem cells are extremely rare within bone marrow (~1 in 10^6^ cells), and while much progress has been made, methodologies allowing for their purification have remained elusive (reviewed in Refs. 30 and 31). Thus, major advances will have to be made in hemopoietic stem cell purification technologies to subsequently develop a clinical protocol for inhibiting IDDM through the use of APC expressing dominantly protective MHC class II gene products.

In conclusion, we have found that the induction of IDDM resistance in NOD mice by transgenic restoration of H2-E MHC class II expression is mediated by multiple subtypes of APC through different, but additive, mechanisms. Only when H2-E is expressed on most all B lymphocytes does this APC subset lose some pathogenic function(s) normally necessary for IDDM development. In contrast, H2-E expression on some critical threshold level of macrophages/DC (~50%) in NOD mice results in the induction of an active immunoregulatory process that functionally suppresses, but does not anergize, autoreactive diabetogenic T cells. Hence, macrophages/DC would appear to be the APC subset that represents the most rational target for therapies that are designed to inhibit IDDM development through the expression of dominantly protective MHC class II alleles.

References


Quantitative thresholds of MHC class II I-E expressed on hematopoietically derived APC in transgenic NOD/Lt mice determine level of diabetes resistance and indicate mechanism of protection. J. Immunol. 157:1279.


