Original Article

Cellular Expression Requirements for Inhibition of Type 1 Diabetes by a Dominantly Protective Major Histocompatibility Complex Haplotype

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The $H2^{g7}$ (K^d, A^{g7}, E^{null}, and D^b) major histocompatibility complex (MHC) is the primary genetic contributor to type 1 diabetes in NOD mice. NOD stocks congenically expressing other MHC haplotypes such as $H2^{nb1}$ (K^b, A^{nb1}, E^k, and D^b) in a heterozygous state are type 1 diabetes resistant. Hematopoietically derived antigen-presenting cells (APCs) expressing $H2^{nb1}$ MHC molecules delete or inactivate autoreactive diabetogenic T-cells. Thus, provided a relatively benign preconditioning protocol is ultimately developed, hematopoietic chimerization by APCs expressing dominantly protective MHC molecules could conceivably provide a means for type 1 diabetes prevention in humans. Before hematopoietic chimerization can be considered for type 1 diabetes prevention, it must be determined what subtype(s) of APCs (B-cells, macrophages, and/or dendritic cells) expressing protective MHC molecules most efficiently inhibit disease, as well as the engraftment level they must achieve to accomplish this. These issues were addressed through analyses of NOD background bone marrow chimeras in which $H2^{nb1}$ molecules were selectively expressed on variable proportions of different APC subtypes. While a modest B-cell effect was observed, the strongest type 1 diabetes protection resulted from at least 50% of dendritic cells and macrophages expressing $H2^{nb1}$ molecules. At this engraftment level, $H2^{nb1}$-expressing dendritic cells and macrophages mediated virtually complete deletion of a highly pathogenic CD8 T-cell population. ***Diabetes*** **56:424–430, 2007**

Type 1 diabetes in both humans and NOD mice results from T-cell–mediated autoimmune destruction of insulin-producing pancreatic β-cells (1). Multiple susceptibility (*Idd*) genes contribute to type 1 diabetes (1). However, particular combinations of major histocompatibility complex (MHC) region genes interactively provide the primary component of type 1 diabetes susceptibility in both humans and NOD mice (1). Antigen-presenting cells (APCs), which include dendritic cells, macrophages, and B-cells, play essential roles not only in the initiation of a protective immune response against pathogens but also in the prevention of autoimmunity. One mechanism by which APCs normally contribute to the maintenance of immunological tolerance is to mediate the physical deletion or functional inactivation of potentially autoreactive T-cells in both the thymus and in the periphery through presentation of self-antigen–derived peptides bound to MHC molecules (2,3). Therefore, it is not surprising that APC defects appear to contribute to type 1 diabetes development in both humans and NOD mice. Indeed, studies in NOD mice indicate that homozygous expression of this strain's unusual $H2^{g7}$ MHC haplotype (K^d, A^{g7}, E^{null}, and D^b), coupled with contributions from other *Idd* genes, gives rise to APCs that are unable to mediate tolerogenic processes normally blocking the development or functional activation of autoreactive diabetogenic T-cells (rev. in 1).

While not the sole pathogenic component, a key MHC-encoded diabetogenic feature of NOD APCs is homozygous expression of H2-A^{g7} but not H2-E class II molecules. This was demonstrated by the finding that type 1 diabetes development is inhibited in a dominant fashion in NOD stocks carrying transgenes encoding H2-A variants from MHC haplotypes other than $H2^{g7}$ or that restore H2-E expression (4–7). Type 1 diabetes is also dominantly inhibited in NOD congenic stocks that express, in a heterozygous state, another MHC haplotype in conjunction with $H2^{g7}$ (8–11). Interestingly, type 1 diabetes resistance elicited by non-$H2^{g7}$ MHC haplotypes congenically introduced to the NOD background interactively results from effects mediated by class I and II molecules that block the development or function of pathogenic T-cells (5,12–14). For example, while β-cell–autoreactive CD8 T-cells of the AI4 clonotype are not deleted when maturing in the presence of K^b MHC class I–expressing APCs, they become functionally anergic (14). Using hematopoietic chimerization approaches, we previously found that when transgenically encoded H2-E is the sole type 1 diabetes–resistant MHC molecule expressed on APCs in NOD mice, diabetogenic T-cells still develop but are functionally inhibited through a peripheral regulatory mechanism (5). On the other hand, hematopoietic chimerization studies have also demonstrated that AI4 T-cells are deleted or permanently anergized when forced to mature in an environment where all APC express the multiple gene products encoded by the type 1 diabetes–protective $H2^{nb1}$ MHC haplotype (K^b, A^{nb1}, E^k, and D^b) (14). Thus, as APCs express a broader array of diabetes-resistant MHC genes, they acquire the ability to activate increasingly diverse

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APC, antigen-presenting cell; MHC, major histocompatibility complex; TCR, T-cell receptor.

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tolerogenic mechanisms that limit both the development and function of β-cell–autoreactive T-cells.

Similar to the case in NOD-related mouse stocks, certain MHC molecules, such as the DQ6 class II variant, also provide dominant type 1 diabetes resistance in humans (rev. in 15). Thus, provided that a relatively benign preconditioning protocol could ultimately be developed, allowing it to be done safely, hematopoietic chimerization giving rise to APCs expressing multiple types of dominantly protective MHC molecules could provide a means for preventing progression to type 1 diabetes in at-risk individuals. Indeed, while using mostly high-dose irradiation–preconditioning protocols, multiple studies have demonstrated that reconstitution with bone marrow from resistant strains can block type 1 diabetes development in NOD mice (16–22). However, even if a benign preconditioning regimen is ultimately developed, before allogeneic hematopoietic chimerization can be considered as a type 1 diabetes prevention therapy in humans, it must be determined what subtype(s) of APCs expressing protective MHC molecules most efficiently inhibit pathogenic T-cell responses, as well as the engraftment level necessary to achieve this. In this regard, it would be highly significant if B-cells efficiently elicit MHC-mediated type 1 diabetes–protective effects, as they represent the APC subtype that can be most readily isolated in large numbers from peripheral blood and thus obviate the need for the more problematical donation of bone marrow. Thus, in this study, we assessed what APC subtype(s) mediate the array of type 1 diabetes–protective effects elicited by multiple genes within the *H2*^{nb1} MHC haplotype and the engraftment levels they must achieve to accomplish this.

RESEARCH DESIGN AND METHODS

NOD/LtDvs mice are maintained at The Jackson Laboratory by brother-sister mating. NOD mice made B-cell deficient by a functionally disrupted *Igμ* allele (designated NOD.*Igμ*^{null}) are maintained at the N10 backcross generation and have been previously described (23). Type 1 diabetes–resistant NOD mice congenic for the NON strain–derived *H2*^{nb1} haplotype are maintained at the N21 backcross generation and have also been described (9). These latter two stocks were crossed to generate a B-cell–deficient NOD stock also homozygous for the *H2*^{nb1} MHC haplotype (designated NOD.*H2*^{nb1}.*Igμ*^{null}). Development of a NOD stock transgenically expressing the rearranged T-cell receptor (TCR) α (Vα8) and β (Vβ2) chain genes from the β-cell–autoreactive CD8 T-cell clone AI4 (designated NOD.*AI4*) has also been previously described (24).

Generation of mixed bone marrow/B-cell chimeras. Female (NOD.*Igμ*^{null} × NOD.*H2*^{nb1}.*Igμ*^{null})F1 mice at 4–6 weeks of age were lethally irradiated (1300R from a ^{137}Cs source) and reconstituted as previously described (10) with 5×10^6 of the indicated T-cell–depleted bone marrow cells. In some experiments, 5×10^6 T-cell–depleted bone marrow cells were admixed with 5×10^6 purified B-cells as indicated. Splenic B-cells were purified using the previously described magnetic bead system (25). The purity of B-cells was >92%, as determined by flow cytometry described below.

Assessment of diabetes development. Type 1 diabetes was assessed by weekly monitoring of glycosuric levels with Ames Diastrix (Bayer, Elkhart, NJ), with disease onset defined by values ≥3.

Histological analysis of insulitis development. The indicated bone marrow–chimeric mice remaining diabetes free for 26 weeks postreconstitution were assessed for insulitis development. Mean insulitis scores were determined as previously described (26). Briefly, pancreata were fixed in Bouin's solution and sectioned at three nonoverlapping levels. Granulated β-cells were stained with aldehyde fuchsin and leukocytes with a hematoxylin and eosin counterstain. Islets (at least 20 per mouse) were individually scored as follows: 0, no lesions; 1, peri-insular leukocytic aggregates, usually periductal infiltrates; 2, <25% islet destruction; 3, >25% islet destruction; and 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.

Analyses of B-cell reconstitution, bone marrow chimerism levels, and the frequency of AI4 T-cells. Flow cytometry was used to determine the levels of B-cell reconstitution, bone marrow chimerism, and AI4 T-cells in recipient mice. B-cells were identified by costaining with the B220-specific antibody (clone RA3-6B2) and polyclonal anti-mouse immunoglobulin. Origins of dendritic cells were detected with antibodies specific for CD11c (clone HL3), the K^d (NOD type) MHC class I variant (clone SF1-1.1), and the K^b (NOD.*H2*^{nb1} type) MHC class I variant (clone 28-13-3). To determine the frequency of AI4 T-cells, thymocytes and splenocytes were stained with antibodies specific for CD8 (clone 53-6.7), CD4 (clone GK1.5), and the TCR Vα8 component (clone B21.14).

RESULTS

Expression of H2^{nb1} molecules solely on dendritic cells/macrophages strongly inhibits type 1 diabetes development. Normally resistant (NOD × NOD.*H2*^{nb1})F1 mice develop type 1 diabetes if irradiated and reconstituted with NOD bone marrow (10). However, when reconstituted with a 1:1 mixture of NOD and NOD.*H2*^{nb1} bone marrow, stable mixed chimerism results, and such F1 hybrids are now protected from type 1 diabetes (10). Subsequent studies indicated that H2^{nb1}-expressing APCs mediated the deletion or permanent inactivation of autoreactive diabetogenic T-cells that normally develop from precursors in NOD bone marrow (27). An unresolved issue was if H2^{nb1} molecules needed to be expressed on all APC subtypes (dendritic cells, macrophages, and B-cells) to mediate the deletion or permanent inactivation of a sufficient array of β-cell–autoreactive T-cell clonotypes to prevent type 1 diabetes development. To do this, we adapted a previously described bone marrow–chimeric system that allows T-cells to mature and function in an environment where expression of *H2*^{nb1} is restricted to dendritic cells/macrophages or B-cells (26).

We first generated (NOD.*Igμ*^{null} × NOD.*H2*^{nb1}.*Igμ*^{null})F1 mice and tested if type 1 diabetes would develop when reconstituted with NOD.*Igμ*^{null} bone marrow and purified NOD B-cells. In this case, all APCs express only the type 1 diabetes–susceptible *H2*^{g7} haplotype, but nonhematopoietically derived cells heterozygously express both H2^{g7} and H2^{nb1} molecules. As shown in Table 1, >60% of the recipients became diabetic by 26 weeks post–bone marrow reconstitution. These results confirmed the previous observation (10) that expression of H2^{nb1} MHC molecules on nonhematopoietic cells is not sufficient to block type 1 diabetes development.

We then tested if *H2*^{nb1} expression solely on dendritic cells/macrophages is capable of suppressing type 1 diabetes. This was done by reconstituting lethally irradiated (NOD.*Igμ*^{null} × NOD.*H2*^{nb1}.*Igμ*^{null})F1 females with NOD.*H2*^{nb1}.*Igμ*^{null} bone marrow admixed with purified NOD B-cells. As summarized in Table 1, none of these recipients became diabetic by 26 weeks postengraftment. It should be noted that in these bone marrow chimeras, the positive selection of T-cells could be mediated by either H2^{g7} and H2^{nb1} MHC molecules (class I: K^d, K^b, or D^b; class II: A^{g7}, A^{nb1}, or E^k) expressed on non–hematopoietically derived thymic epithelial cells. However, with the exception of D^b, all other H2^{nb1}- or H2^{g7}-specific MHC class I/II molecules were solely expressed by either the dendritic cell/macrophage or B-cell subsets of hematopoietically derived APCs, respectively, available to activate T-cell effector function in the periphery. Therefore, complete type 1 diabetes resistance in these particular chimeras could be due to the lack of K^d- and/or A^{g7}-expressing dendritic cells/macrophages, which may be required to fully activate β-cell–autoreactive T-cells restricted to these *H2*^{g7}-encoded MHC molecules. To rule out this possibility, we reconstituted lethally irradiated (NOD.*Igμ*^{null} ×

TABLE 1
$H2^{nb1}$ MHC molecules more strongly suppress type 1 diabetes development when solely expressed on dendritic cells/macrophages than the B-cell subset of APCs*

Cell types used to reconstitute recipient mice	n	Proportion of B-cells in spleen (%)†	Diabetes incidence at 26 weeks posttransfer (%)	P value‡
NOD.*Ig*μ^{null} *BM + NOD B-cells*	21	33.05 ± 1.86	61.9	
NOD.*H2*^{nb1}.*Ig*μ^{null} *BM + NOD B-cells*	10	31.22 ± 4.07	0	0.0011
(NOD.*Ig*μ^{null} × *NOD.H2*^{nb1}.*Ig*μ^{null})*F1 BM +NOD B-cells*	10	27.91 ± 2.11	0	0.0011
NOD.*Ig*μ^{null} *BM + NOD.H2*^{nb1} *B-cells*	10	13.24 ± 1.6	20	0.0291

Data are means ± SE unless otherwise indicated. *Female (NOD.*Ig*μ^{null} × *NOD.H2*^{nb1}.*Ig*μ^{null}*)F1* mice were lethally irradiated at 4–6 weeks of age and reconstituted with indicated bone marrow (BM; 5×10^6) and purified B-cells (5×10^6). Type 1 diabetes development was monitored for a period of 26 weeks posttransfer. †Proportion of B-cells (identified by anti-B220 and -Ig antibodies) was determined at either the onset of diabetes or the end of the study. ‡Statistical analysis by χ^2 (versus NOD.*Ig*μ^{null} bone marrow and NOD B-cell recipients).

NOD.*H2*^{nb1}.*Ig*μ^{null})F1 recipient mice with syngeneic bone marrow admixed with purified NOD B-cells. In this case, the same $H2^{g7}$ MHC molecules available to positively select T-cells in the thymus are also coexpressed with *H2*^{nb1}-encoded variants on all dendritic cells/macrophages in the periphery. As shown in Table 1, such recipient mice were still highly protected from type 1 diabetes. These results indicated that heterozygous expression of $H2^{g7}$ MHC molecules on dendritic cells/macrophages was not sufficient to break type 1 diabetes resistance conferred by *H2*^{nb1}-encoded gene products on the same cells. In addition to protection from overt diabetes, insulitis was also significantly reduced by the presence of *H2*^{nb1}-expressing dendritic cells/macrophages (Fig. 1). Therefore, heterozygous expression of *H2*^{nb1}-encoded genes solely by the dendritic cell/macrophage lineages of APCs is sufficient to strongly inhibit type 1 diabetes development in NOD background mouse stocks.

$H2^{nb1}$ molecules provide weaker type 1 diabetes–protective effects when expressed solely on B-cells rather than dendritic cells/macrophages. We next tested if *H2*^{nb1} expression exclusively by all B-cells was sufficient to suppress type 1 diabetes. This was done by reconstituting lethally irradiated (NOD.*Ig*μ^{null} × NOD.*H2*^{nb1}.*Ig*μ^{null})F1 females with NOD.*Ig*μ^{null} bone marrow admixed with purified NOD.*H2*^{nb1} B-cells. Expression of $H2^{nb1}$ MHC molecules solely on B-cells was also capable of retarding type 1 diabetes development and reducing insulitis. However, B-cells did so less efficiently than dendritic cells/macrophages (Table 1 and Fig. 1). It should be noted that the extent to which $H2^{nb1}$-expressing B-cells were able to block type 1 diabetes development in the F1 recipients did not correlate with their level of engraftment. This is illustrated by the fact that the proportion of $H2^{nb1}$-expressing B-cells among splenic leukocytes in the 2/10 F1 recipients that did develop type 1 diabetes was 5.8 and 18.6%, while the overall group mean repopulation level was 13.2%.

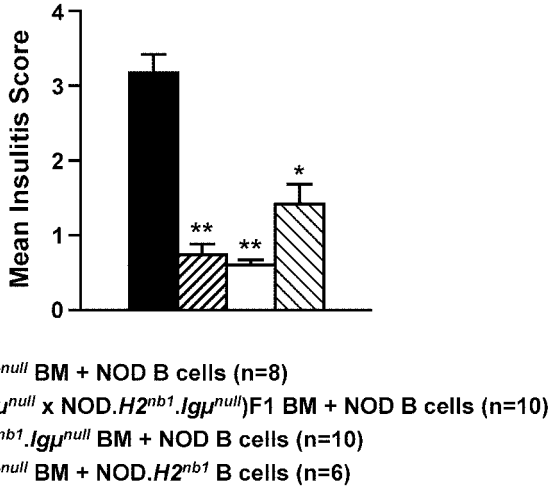

FIG. 1. Expression of $H2^{nb1}$ MHC molecules solely on dendritic cells/macrophages or B-cells reduces insulitis. Female (NOD.*Ig*μ^{null} × NOD.*H2*^{nb1}.*Ig*μ^{null})F1 mice were lethally irradiated at 4–6 weeks of age and reconstituted with indicated bone marrow (BM) and purified B-cells. Insulitis was determined for nondiabetic recipients at 26 weeks postreconstitution. The results are presented as mean insulitis score ± SE. *$P < 0.05$, significantly lower than NOD. *Ig*μ^{null} bone marrow/NOD B-cell recipients (Wilcoxon's rank-sum test). **$P < 0.05$, significantly lower than NOD.*Ig*μ^{null} bone marrow/NOD B-cell and NOD.*Ig*μ^{null} bone marrow/NOD.*H2*^{nb1} B-cell recipients (Wilcoxon's rank-sum test).

To independently induce efficient type 1 diabetes resistance, ~50% of dendritic cells/macrophages must express $H2^{nb1}$ molecules. We next determined what proportion of the dendritic cell/macrophage subset of APCs must express $H2^{nb1}$ molecules to independently block type 1 diabetes development in an efficient fashion. This was done by reconstituting lethally irradiated (NOD.*Ig*μ^{null} × NOD.*H2*^{nb1}.*Ig*μ^{null})F1 females with different proportions of admixed syngeneic and NOD bone marrow. In this case, all B-cells are of NOD origin, and different levels of *H2*^{nb1}-expressing dendritic cells/macrophages are present in various groups of recipient mice. The proportion of dendritic cells/macrophages displaying $H2^{nb1}$ molecules was determined by analyzing K^b expression on $CD11c^+$ cells (Fig. 2). As shown in Fig. 3*A*, compared with controls reconstituted with NOD bone marrow alone, type 1 diabetes development was most efficiently suppressed in recipients in which $H2^{nb1}$ molecules were independently expressed on ≥50% of the dendritic cell/macrophage lineages of APCs. While also developing at levels that statistical analyses indicated to be significantly suppressed compared with controls reconstituted with NOD bone marrow alone, a more modest type 1 diabetes–protective effect was observed in recipients where the proportion of dendritic cells/macrophages independently expressing $H2^{nb1}$ molecules did not exceed 50% (Fig. 3*A*). In those recipients in which the proportion of *H2*^{nb1}-expressing dendritic cells/macrophages did not exceed 50%, the reconstitution levels of such APCs did not correlate with type 1 diabetes susceptibility or resistance (Fig. 3*B*). Furthermore, the finding that the engraftment levels of $H2^{nb1}$-positive dendritic cells/macrophages must approach 50% to efficiently inhibit type 1 diabetes devel-

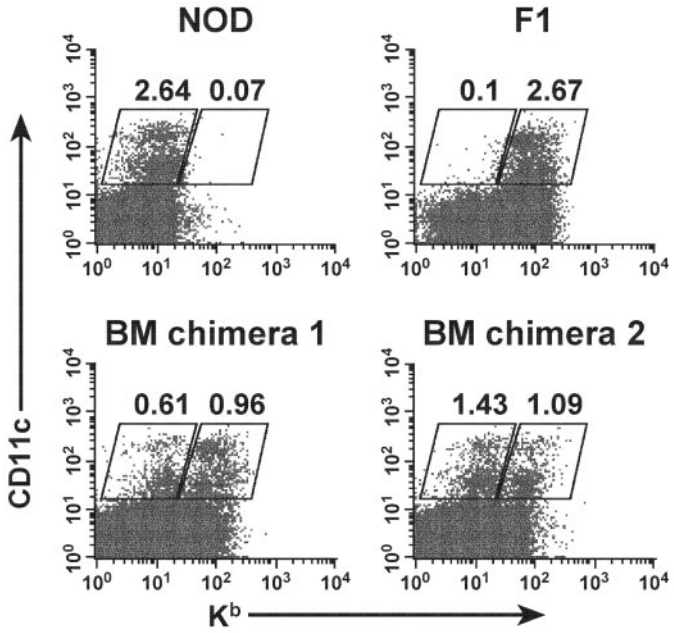

FIG. 2. Expression of *H2*^{nb1}-encoded K^b MHC molecules defines the origin of dendritic cells in bone marrow (BM) chimeras. Flow cytometry was used to distinguish dendritic cells (CD11c$^+$) of NOD or (NOD.*Ig*μ^{null} × NOD.*H2*^{nb1}.*Ig*μ^{null})F1 origin by the respective absence or presence of K^b expression. Examples of two bone marrow chimeras are shown.

opment indicates that low-level contamination by these myeloid APCs was unlikely to be a protective factor in the studies that assessed the ability of highly purified B-cells (>92%) expressing the same MHC molecules to block disease onset.

H2^{nb1}-expressing dendritic cells/macrophages engrafted at levels efficiently inducing type 1 diabetes resistance mediate the virtually complete deletion of a β-cell–autoreactive CD8 T-cell clonotype. *H2*^{nb1}-expressing APCs can mediate the deletion to variable degrees of at least some β-cell–autoreactive T-cell clonotypes normally contributing to type 1 diabetes in NOD mice (13,14). We previously demonstrated that *H2*^{nb1} expression on 15–40% of dendritic cells/macrophages resulted in partial, but incomplete, deletion in both the thymus and spleen of the important diabetogenic CD8 T-cell clonotype AI4 (27). However, as shown earlier, H2^{nb1} molecules must be expressed by at least 50% of the dendritic cell/macrophage lineages of APCs to efficiently inhibit type 1 diabetes development. Thus, we reasoned that an evaluation of the level to which AI4 T-cells develop when forced to differentiate under conditions where the proportion of *H2*^{nb1}-expressing dendritic cells/macrophages exceeded 50% could provide insight regarding the extent that such APCs mediate strong type 1 diabetes resistance by inducing the deletion of pathogenic effectors versus the activation of other potential regulatory mechanisms.

Lethally irradiated (NOD.*Ig*μ^{null} × NOD.*H2*^{nb1}.*Ig*μ^{null})F1 mice were reconstituted with a 2:1 mixture of syngeneic F1 and NOD.*AI4* bone marrow. At 8 weeks postreconstitution, 57–73% of the dendritic cells/macrophages in these chimeras heterozygously expressed H2^{nb1} molecules (all B-cells were homozygous for *H2*^{g7}). Control chimeras consisted of the same type of F1 recipients reconstituted with a 2:1 mixture of standard NOD and NOD.*AI4* bone marrow. All APCs in the control chimeras homozygously expressed H2^{g7} molecules. AI4 T-cells were identified in the control and test chimeras by coexpression of CD8 and the TCR Vα8 element (Fig. 4*A*). Compared with their levels in controls, the numbers of CD8 single-positive thymocytes that expressed the AI4 TCR were reduced by >93%

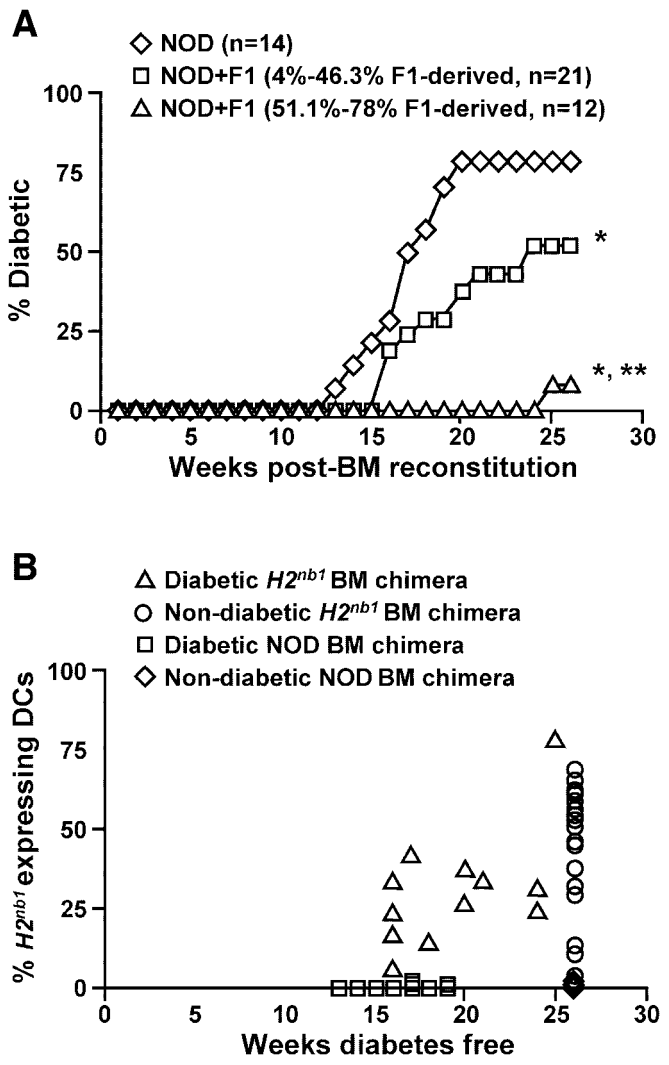

FIG. 3. To independently induce efficient type 1 diabetes resistance, ~50% of dendritic cells/macrophages must express *H2*^{nb1} molecules. Female (NOD.*Ig*μ^{null} × NOD.*H2*^{nb1}.*Ig*μ^{null})F1 mice were lethally irradiated at 4–6 weeks of age and reconstituted with NOD bone marrow (BM) alone or admixed with syngeneic (F1) bone marrow. The levels of F1 bone marrow–derived dendritic cells/macrophages were determined by fluorescence-activated cell sorting as described in RESEARCH DESIGN AND METHODS. *A*: Mixed bone marrow chimeras are grouped based on the chimerization level (> or <50% F1 bone marrow derived) and assessed for type 1 diabetes development. **P* < 0.05, significantly different from NOD bone marrow–only recipients (Kaplan-Meier log-rank analysis). ***P* < 0.05, significantly different from NOD and (NOD.*Ig*μ^{null} × NOD.*H2*^{nb1}.*Ig*μ^{null})F1 bone marrow recipients with <50% F1-derived dendritic cells/macrophages (Kaplan-Meier log-rank analysis). *B*: Unless exceeding 50%, the reconstitution levels of H2^{nb1}-expressing dendritic cells/macrophages do not correlate with type 1 diabetes susceptibility or resistance. Data depict the length of time postreconstitution that the same chimeras shown in *A* remained free of type 1 diabetes as a function of levels of H2^{nb1}-expressing dendritic cells/macrophages. The experiment was terminated at 26 weeks postreconstitution. △ and ○ represent recipients with varying levels of H2^{nb1}-expressing dendritic cells/macrophages that did or did not progress to overt type 1 diabetes, respectively. □ and ◇ represent controls reconstituted with NOD bone marrow only that did or did not progress to overt type 1 diabetes, respectively.

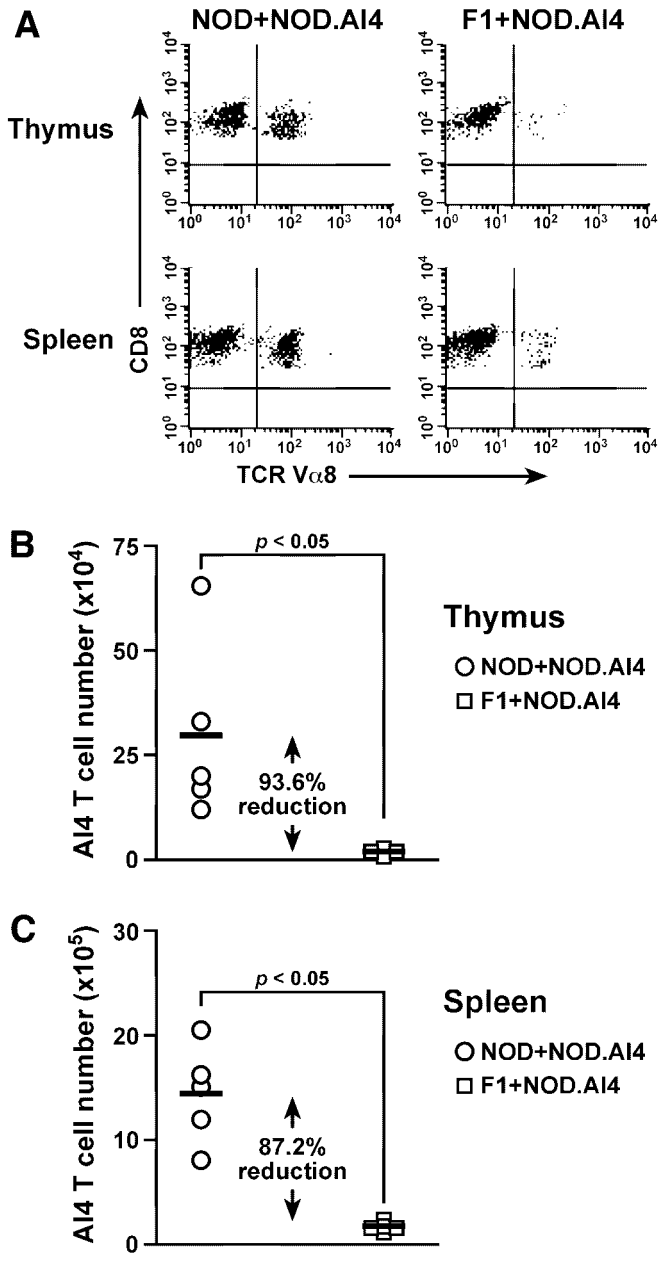

FIG. 4. *H2*^{nb1}-expressing dendritic cell/macrophage engrafted at levels efficiently inducing type 1 diabetes resistance mediate the virtually complete deletion of a β-cell–autoreactive CD8 T-cell clonotype. Female (NOD.*Igμ*^{null} × NOD.*H2*^{nb1}.*Igμ*^{null})F1 mice were lethally irradiated at 4–6 weeks of age and reconstituted with bone marrow from syngeneic and NOD.*AI4* TCR transgenic donors at a ratio of 2:1. Controls were the same type of F1 mice reconstituted with NOD and NOD.*AI4* TCR transgenic bone marrow at a ratio of 2:1. At 8 weeks postreconstitution, the proportions of dendritic cells derived from each donor bone marrow type as well as the percentages and numbers of CD8 single-positive thymocytes and splenocytes that expressed the AI4 TCR were assessed. *A*: Representative fluorescence-activated cell sorting profiles of thymocytes (gated on CD4$^-$CD8$^+$) and splenocytes (gated on CD8$^+$) expressing the AI4 TCR Vα8 chain. *B*: Numbers of AI4 CD8 SP thymocytes. *C*: Numbers of AI4 T-cells in the spleen. Statistical analyses in *B* and *C* were carried out by Wilcoxon's rank-sum test.

in the chimeras in which the proportion of *H2*^{nb1}-expressing dendritic cells/macrophages exceeded 50% (Fig. 4*B*). This carried over to the periphery, since numbers of AI4 T-cells in the spleens of the test chimeras were also reduced by >87% compared with their levels in controls (Fig. 4*C*). We previously found that some CD4 T-cells are still generated in NOD.*AI4* mice but that their development and function is controlled by endogenously derived rather than coexpressed transgenic TCR molecules (24). This probably explains why peripheral levels of CD4 T-cells expressing the AI4 TCR were not influenced by the presence of *H2*^{nb1}-expressing APCs (data not shown). The array of autoreactive T-cell responses that aberrantly develop and cause type 1 diabetes in human patients and NOD mice are clearly normally prevented in most other individuals and mouse strains by multiple regulatory mechanisms (28). However, our current results show that the virtually complete deletion of at least AI4, and perhaps other highly pathogenic T-cell clonotypes, represents one contributory mechanism by which engraftment with a sufficiently high threshold level of dendritic cells/macrophages expressing dominantly protective H2^{nb1} MHC molecules elicits type 1 diabetes resistance in normally susceptible NOD mice.

DISCUSSION

In the current study, we have demonstrated that expression of a disease-protective MHC haplotype (*H2*^{nb1}) solely on either dendritic cells/macrophages or B-cells is capable of inhibiting insulitis and type 1 diabetes development in NOD mice. However, dendritic cells/macrophages accomplish this more efficiently than B-cells. It was also found that their ability to independently inhibit type 1 diabetes development in NOD recipients was most efficient when bone marrow chimerization resulted in at least ~50% of dendritic cells/macrophages expressing *H2*^{nb1} molecules, albeit with lower but still significant protection achieved at lower repopulation levels. One likely explanation why dendritic cells/macrophages provide stronger *H2*^{nb1}-mediated type 1 diabetes–protective effects than B-cells is that the former myeloid lineages represent the primary type of APCs available in the thymus to mediate negative selection. Indeed, when dendritic cells and macrophages are the only types of APCs to express *H2*^{nb1} molecules and have engrafted at levels that most efficiently inhibit type 1 diabetes development in NOD recipients, autoreactive CD8 T-cells of the AI4 clonotype undergo virtually complete intrathymic deletion. On the other hand, while they do so in a less efficient manner than the dendritic cell/macrophage lineages of APCs, our results indicate that *H2*^{nb1}-expressing B-cells are also able to significantly reduce insulitis and inhibit progression to type 1 diabetes in NOD mice most likely through peripheral tolerance induction mechanisms. These collective results suggest that perhaps due to an ability to activate a wider array of immunoregulatory mechanism than when limited to a single lineage, protective MHC haplotypes may most efficiently inhibit type 1 diabetes development when expressed by all types of APCs.

Certain HLA molecules, such as the DQ6 class II variant, also provide dominant type 1 diabetes resistance in humans (rev. in 15). Therefore, bone marrow chimerization with donor cells expressing protective HLA alleles represents an attractive treatment for prevention of type 1 diabetes in humans at risk for this disease or allowing reversal of hyperglycemia by cotransplantation of donor-matched pancreatic islets. However, clinical application of bone marrow chimerization protocols for type 1 diabetes prevention or reversal has been precluded by the risks associated with this procedure. One adverse outcome is the development of graft versus host disease. Another

concern is the toxicity caused by high-dose irradiation, which is required to precondition recipients for donor bone marrow engraftment.

Previous studies (20–22) have successfully used preconditioning protocols not including a lethal dose of irradiation to achieve allogeneic bone marrow chimerization as a means to block type 1 diabetes development or reverse the disease in NOD mice when combined with donor-matched islet transplantation. However, in all cases, initially established mixed bone marrow chimerism could not be maintained, as virtually all hematopoietic cells converted to donor type at some point postreconstitution (20–22). While type 1 diabetes can be prevented, full allogeneic bone marrow chimerism also predisposes recipients to an undesirable immunocompromised state (27). We recently reported a new conditioning protocol that includes a low nonlethal irradiation dose (600 R) and injection of CD154 (i.e., CD40L)-blocking and CD8 T-cell–depleting antibodies (27). This protocol induced a stable partial state of allogeneic hematopoietic chimerism (~50% donor derived) and completely prevented type 1 diabetes development in NOD mice. Most importantly, the generalized immunosuppression observed in full allogeneic bone marrow–chimerized recipients was avoided (27).

Our new preconditioning regimen, which does not require high-dose irradiation, allowed us to achieve encouraging results, but it did not allow us to establish allogeneic hematopoietic chimerism levels of <50% in NOD recipients (27). As a result, we were forced to use a high-dose irradiation preconditioning regimen to determine the minimal level of engraftment that must be achieved by various subtypes of APC-expressing type 1 diabetes–protective MHC molecules to block disease development in NOD recipients. One potentially significant outcome of these analyses is that the ~50% engraftment threshold at which $H2^{nb1}$-expressing APCs efficiently block type 1 diabetes development in NOD recipients is a level achievable through use of our newly developed preconditioning regimen, which does not require a high lethal dose of irradiation.

In conclusion, we have demonstrated in the NOD mouse model that the spectrum of disease-inhibitory effects that can be elicited by a complete type 1 diabetes–protective MHC haplotype are most readily manifest when its gene products are expressed by the dendritic cell and macrophage subsets of APCs rather than B-cells. In addition, our results indicate that when dendritic cells and macrophages are the only APCs expressing the type 1 diabetes–protective $H2^{nb1}$ haplotype, at least 50% of them have to do so to achieve a maximal level of disease resistance. These results further enhance our knowledge for designing bone marrow transplantation protocols that may ultimately provide a therapeutic means to prevent or reverse type 1 diabetes in humans.

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REFERENCES

1. Serreze DV, Leiter EH: Genes and cellular requirements for autoimmune diabetes susceptibility in nonobese diabetic mice. *Curr Dir Autoimmun* 4:31–67, 2001
2. Walker LS, Abbas AK: The enemy within: keeping self-reactive T cells at bay in the periphery. *Nat Rev Immunol* 2:11–19, 2002
3. Kyewski B, Klein L: A central role for central tolerance. *Annu Rev Immunol* 24:571–606, 2006
4. Singer SM, Tisch R, Yang XD, McDevitt HO: An Abd transgene prevents diabetes in nonobese diabetic mice by inducing regulatory T cells. *Proc Natl Acad Sci U S A* 90:9566–9570, 1993
5. Hanson MS, Cetkovic-Cvrlje M, Ramiya VK, Atkinson MA, Maclaren NK, Singh B, Elliott JF, Serreze DV, Leiter EH: Quantitative thresholds of MHC class II I-E expressed on hemopoietically derived antigen-presenting cells in transgenic NOD/Lt mice determine level of diabetes resistance and indicate mechanism of protection. *J Immunol* 157:1279–1287, 1996
6. Uehira M, Uno M, Kurner T, Kikutani H, Mori K, Inomoto T, Uede T, Miyazaki J, Nishimoto H, Kishimoto T, et al.: Development of autoimmune insulitis is prevented in E alpha d but not in A beta k NOD transgenic mice. *Int Immunol* 1:209–213, 1989
7. Lund T, O'Reilly L, Hutchings P, Kanagawa O, Simpson E, Gravely R, Chandler P, Dyson J, Picard JK, Edwards A, et al.: Prevention of insulin-dependent diabetes mellitus in non-obese diabetic mice by transgenes encoding modified I-A beta-chain or normal I-E alpha-chain. *Nature* 345:727–729, 1990
8. Wicker LS, Miller BJ, Fischer PA, Pressey A, Peterson LB: Genetic control of diabetes and insulitis in the nonobese diabetic mouse: pedigree analysis of a diabetic H-2nod/b heterozygote. *J Immunol* 142:781–784, 1989
9. Prochazka M, Serreze DV, Worthen SM, Leiter EH: Genetic control of diabetogenesis in NOD/Lt mice: development and analysis of congenic stocks. *Diabetes* 38:1446–1455, 1989
10. Serreze DV, Leiter EH: Development of diabetogenic T cells from NOD/Lt marrow is blocked when an allo-H-2 haplotype is expressed on cells of hemopoietic origin, but not on thymic epithelium. *J Immunol* 147:1222–1229, 1991
11. Wicker LS, Appel MC, Dotta F, Pressey A, Miller BJ, DeLarato NH, Fischer PA, Boltz RC Jr, Peterson LB: Autoimmune syndromes in major histocompatibility complex (MHC) congenic strains of nonobese diabetic (NOD) mice: the NOD MHC is dominant for insulitis and cyclophosphamide-induced diabetes. *J Exp Med* 176:67–77, 1992
12. Schmidt D, Amrani A, Verdaguer J, Bou S, Santamaria P: Autoantigen-independent deletion of diabetogenic CD4+ thymocytes by protective MHC class II molecules. *J Immunol* 162:4627–4636, 1999
13. Schmidt D, Verdaguer J, Averill N, Santamaria P: A mechanism for the major histocompatibility complex-linked resistance to autoimmunity. *J Exp Med* 186:1059–1075, 1997
14. Serreze DV, Holl TM, Marron MP, Graser RT, Johnson EA, Choisy-Rossi C, Slattery RM, Lieberman SM, DiLorenzo TP: MHC class II molecules play a role in the selection of autoreactive class I-restricted CD8 T cells that are essential contributors to type 1 diabetes development in nonobese diabetic mice. *J Immunol* 172:871–879, 2004
15. Nepom GT, Kwok WW: Molecular basis for HLA-DQ associations with IDDM. *Diabetes* 47:1177–1184, 1998
16. Ikehara S, Ohtsuki H, Good RA, Asamoto H, Nakamura T, Sekita K, Muso E, Tochino Y, Ida T, Kuzuya H, et al.: Prevention of type I diabetes in nonobese diabetic mice by allogenic bone marrow transplantation. *Proc Natl Acad Sci U S A* 82:7743–7747, 1985
17. LaFace DM, Peck AB: Reciprocal allogeneic bone marrow transplantation between NOD mice and diabetes-nonsusceptible mice associated with transfer and prevention of autoimmune diabetes. *Diabetes* 38:894–901, 1989
18. Li H, Kaufman CL, Boggs SS, Johnson PC, Patrene KD, Ildstad ST: Mixed allogeneic chimerism induced by a sublethal approach prevents autoimmune diabetes and reverses insulitis in nonobese diabetic (NOD) mice. *J Immunol* 156:380–388, 1996
19. Mathieu C, Casteels K, Bouillon R, Waer M: Protection against autoimmune diabetes in mixed bone marrow chimeras: mechanisms involved. *J Immunol* 158:1453–1457, 1997
20. Seung E, Iwakoshi N, Woda BA, Markees TG, Mordes JP, Rossini AA, Greiner DL: Allogeneic hematopoietic chimerism in mice treated with sublethal myeloablation and anti-CD154 antibody: absence of graft-versus-host disease, induction of skin allograft tolerance, and prevention of recurrent autoimmunity in islet-allografted NOD/Lt mice. *Blood* 95:2175–2182, 2000
21. Nikolic B, Takeuchi Y, Leykin I, Fudaba Y, Smith RN, Sykes M: Mixed hematopoietic chimerism allows cure of autoimmune diabetes through allogeneic tolerance and reversal of autoimmunity. *Diabetes* 53:376–383, 2004
22. Ildstad ST, Chilton PM, Xu H, Domenick MA, Ray MB: Preconditioning of

NOD mice with anti-CD8 mAb and costimulatory blockade enhances chimerism and tolerance and prevents diabetes, while depletion of alpha beta-TCR+ and CD4+ cells negates the effect. *Blood* 105:2577–2584, 2005
23. Silveira PA, Johnson E, Chapman HD, Bui T, Tisch RM, Serreze DV: The preferential ability of B lymphocytes to act as diabetogenic APC in NOD mice depends on expression of self-antigen-specific immunoglobulin receptors. *Eur J Immunol* 32:3657–3666, 2002
24. Graser RT, DiLorenzo TP, Wang F, Christianson GJ, Chapman HD, Roopenian DC, Nathenson SG, Serreze DV: Identification of a CD8 T cell that can independently mediate autoimmune diabetes development in the complete absence of CD4 T cell helper functions. *J Immunol* 164:3913–3918, 2000
25. Serreze DV, Fleming SA, Chapman HD, Richard SD, Leiter EH, Tisch RM: B lymphocytes are critical antigen-presenting cells for the initiation of T cell-mediated autoimmune diabetes in nonobese diabetic mice. *J Immunol* 161:3912–3918, 1998
26. Johnson EA, Silveira P, Chapman HD, Leiter EH, Serreze DV: 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. *J Immunol* 167:2404–2410, 2001
27. Serreze DV, Osborne MA, Chen YG, Chapman HD, Pearson T, Brehm MA, Greiner DL: Partial versus full allogeneic hematopoietic chimerization is a preferential means to inhibit type 1 diabetes as the latter induces generalized immunosuppression. *J Immunol* 177:6675–6684, 2006
28. Anderson MS, Bluestone JA: The NOD mouse: a model of immune dysregulation. *Annu Rev Immunol* 23:447–485, 2005