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Identification of a CD8 T Cell That Can Independently Mediate Autoimmune Diabetes Development in the Complete Absence of CD4 T Cell Helper Functions[1]

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Previous work has indicated that an important component for the initiation of autoimmune insulin-dependent diabetes mellitus (IDDM) in the NOD mouse model entails MHC class I-restricted CD8 T cell responses against pancreatic β cell Ags. However, unless previously activated in vitro, such CD8 T cells have previously been thought to require helper functions provided by MHC class II-restricted CD4 T cells to exert their full diabetogenic effects. In this study, we show that IDDM development is greatly accelerated in a stock of NOD mice expressing TCR transgenes derived from a MHC class I-restricted CD8 T cell clone (designated AI4) previously found to contribute to the earliest preclinical stages of pancreatic β cell destruction. Importantly, these TCR transgenic NOD mice (designated NOD.AI4$\alpha\beta$ Tg) continued to develop IDDM at a greatly accelerated rate when residual CD4 helper T cells were eliminated by introduction of the *scid* mutation or a functionally inactivated *CD4* allele. In a previously described stock of NOD mice expressing TCR transgenes derived from another MHC class I-restricted β cell autoreactive T cell clone, IDDM development was retarded by elimination of residual CD4 T cells. Hence, there is variability in the helper dependence of CD8 T cells contributing to the development of autoimmune IDDM. The AI4 clonotype represents the first CD8 T cell with a demonstrated ability to progress from a naive to functionally activated state and rapidly mediate autoimmune IDDM development in the complete absence of CD4 T cell helper functions. ***The Journal of Immunology,*** **2000, 164: 3913–3918.**

Insulin-dependent diabetes mellitus (IDDM)[4] in both humans and NOD mice results from T cell-mediated autoimmune destruction of pancreatic β cells (1–3). While under polygenic control in both genera, the primary genetic components contributing to IDDM susceptibility reside within specific MHC haplotypes (4–6). In humans, IDDM susceptibility is largely provided by specific combinations of HLA-DQ and DR MHC class II alleles (7). Similarly, the development of autoimmune IDDM in NOD mice requires that the rare H2-A^{g7} MHC class II gene product (homologue of human DQ) be homozygously expressed in the absence of H2-E MHC class II molecules (homologue of human DR) (8–12). In contrast, since the K^d and D^b class I gene products encoded within the *H2*^{g7} MHC haplotype of NOD mice represent common variants also found in numerous strains that do not develop autoimmunity, it was originally thought that they would not contribute to IDDM development. However, a number of studies have indicated that CD8 T cell responses restricted to the common K^d and/or D^b MHC class I variants are required for IDDM development. First, a combination of both CD4 and CD8 cells are required to adoptively transfer IDDM from young prediabetic NOD donors to lymphocyte deficient NOD-*scid* recipients (13). In addition, neither IDDM nor insulitis develops in NOD mice made deficient in MHC class I expression and CD8 T cells by the presence of an inactivated β_2-microglobulin allele (designated NOD.*β2m*^{null} mice) (14, 15). Subsequent studies demonstrated that CD8 T cells restricted to the common $H2^{g7}$ MHC class I gene products are essential contributors to the earliest initiative phases of autoimmune pancreatic β cell destruction leading to IDDM development in NOD mice (16, 17).

Given their indispensable diabetogenic role, it is important to identify the mechanisms that enable MHC class I-restricted β cell-autoreactive CD8 T cells to develop and exert their pathogenic functions. The goal of the present study was to determine whether MHC class I-restricted β cell-autoreactive T cell responses essential to IDDM development in NOD mice could still be generated in the absence of helper functions provided by MHC class II-restricted T cells. This issue was addressed through the use of a newly developed NOD mouse stock that transgenically expresses the TCR from a MHC class I-restricted CD8 clone that contributes to the earliest initiative phases of autoimmune β cell destruction.

Materials and Methods

Mice

NOD/Lt mice are maintained at The Jackson Laboratory by brother-sister mating. Currently, IDDM develops in 90% of female and 63% of male NOD/Lt mice by 1 year of age. Stocks of NOD mice transgenically expressing the rearranged TCR α- and/or β-chain genes from the previously described (17) K^d-restricted β cell-autoreactive CD8 T cell clone AI4 were produced as follows. TCRα and -β shuttle vectors (18, 19) were kindly provided by Dr. Mark Davis (Stanford University, Stanford, CA). The VJ segment of the AI4 TCR α-chain cDNA (Vα8) was amplified by PCR using primers containing appropriate intronic sequences, splice acceptor/

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[4] Abbreviations used in this paper: IDDM, insulin-dependent diabetes mellitus; PBL, peripheral blood leukocyte.

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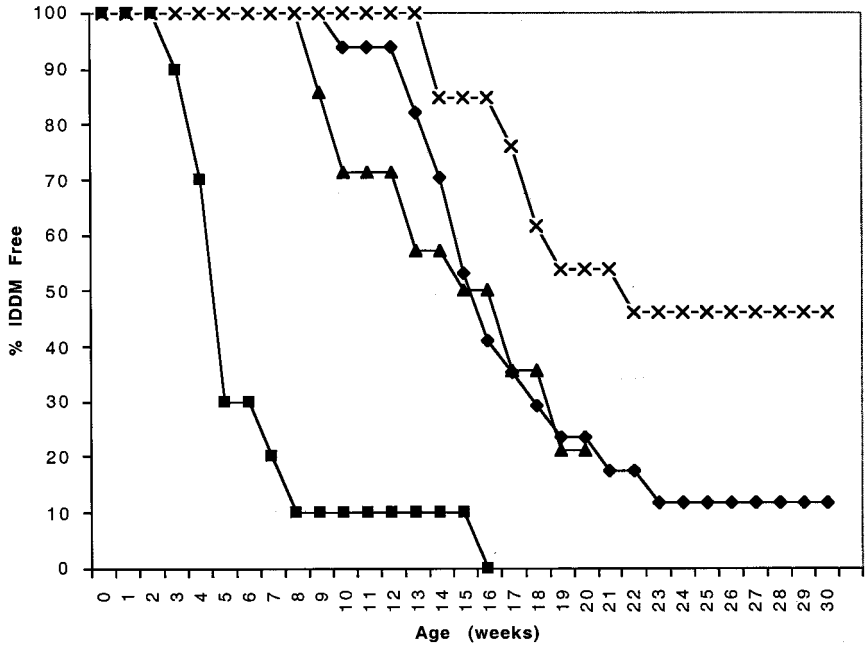

FIGURE 1. Accelerated IDDM development in NOD.AI4$\alpha\beta$ Tg mice. The rates of IDDM development in NOD females separately expressing AI4 TCR α-chain (▲, $n = 14$) or β-chain (**x**, $n = 16$) transgenes, or the two constructs together (■, $n = 10$), were compared with that of nontransgenic controls (♦, $n = 18$).

donor sites, and restriction sites for cloning into the *Xho*I/*Not*I sites of the TCRα shuttle vector. The VDJ segment of the TCR β-chain cDNA (Vβ2) was similarly amplified and cloned into the *Cla*I/*Not*I sites of the TCRβ shuttle vector. Prokaryotic sequences were removed by digestion with *Cla*I/*Sal*I (TCRα) or *Pvu*I/*Sal*I (TCRβ). These transgene constructs were separately injected directly into naturally ovulated NOD/Lt zygotes by the Microinjection Service of The Jackson Laboratory and implanted without prior culture into pseudopregnant (B6 × SJL)F_1 females. Offspring carrying the AI4 TCR α-chain transgene (designated NOD.AI4α Tg) were identified by PCR using the primer set 5′-CTCCGTGACCCAGACAGAAGG-3′ and 5′-TTCCAGTGTTAGCACCAGCCG-3′. Carriers of the AI4 TCR β-chain transgene (designated NOD.AI4β Tg) were identified by PCR using the primer set 5′-GCTGGAGCAAAACCCAAGGTG-3′ and 5′-GTATTTCCTAGCCCCCTGTGTG-3′. TCR transgene expression was confirmed by flow cytometry as described below. NOD mice carrying both the AI4 TCR α- and β-chain transgenes (designated NOD.AI4$\alpha\beta$ Tg mice) were then produced through intercrossing. Previously described IDDM-resistant strains of NOD-*scid* (official designation NOD-*Prkdc*^{scid}) and NOD.β*2m*^{null} mice (official designation NOD.*B2m*^{tm1Unc}) are each maintained at the N11 backcross generation (14, 20). These two strains served as progenitors for two new stocks respectively designated NOD-*scid*.AI4$\alpha\beta$ Tg and NOD-β*2m*^{null}.AI4$\alpha\beta$ Tg. NOD-*scid* homozygotes were outcrossed to mice carrying the AI4 TCR transgenes. The subsequent F_1 progeny were backcrossed to NOD-*scid* to produce offspring homozygous for *scid* and heterozygous for the AI4 TCR transgene constructs. A similar method was used to generate AI4 TCR Tg NOD mice homozygous for the β*2m*^{null} mutation. An N8 backcross stock of NOD mice homozygous for a *CD4*^{null} allele (Ref. 21; official designation *Cd4*^{tm1Knw}), as well as genetic linkage markers delineating all known diabetes susceptibility (*Idd*) loci of NOD origin, was produced by our previously described "speed congenic" approach (22). NOD mice homozygous for the *CD4*^{null} allele and carrying the AI4 TCR transgenes were produced as described above. All mice are housed under specific pathogen-free conditions and allowed free access to food (Agway Diet NIH 31A; Agway, South Henly, MO) and acidified drinking water. NOD-*scid* stocks received trimethoprim-sulfamethoxazole supplement (Sulfatrim; Barre-National, Baltimore, MD) 3 days per wk.

Assessment of diabetes development

Diabetes development in the indicated mice was defined by glycosuric values of ≥3 as assessed with Ames Diastix (kindly supplied by Miles Diagnostics, Elkhart, IN).

T cell subset enumerations

Peripheral blood leukocytes (PBL) from the indicated mice were assessed for CD4 and CD8 T cell levels and the proportion of these that express Tg AI4 TCR elements by multicolor flow cytometric techniques (FACScan; Becton Dickinson, San Jose CA) using the CellQuest 3.0 data reduction system. Total T cells were detected with the TCR$\alpha\beta$-specific mAb H57-597 conjugated to a green fluorescent FITC tag. Total T cells were then further characterized for CD4 expression using the mAb GK1.5 conjugated to the red fluorescent tag Cy3.18-OSu (Cy3; Biological Detection Systems, Pittsburgh, PA) or for CD8 expression with the mAb 53-6.72 conjugated to PE whose red fluorescence intensity can easily be distinguished from that of Cy3. A separate aliquot of PBL from each mouse was assessed by two-color FACS analysis for Tg AI4 α- and/or β-chain expression by respective use of PE- or FITC-conjugated Abs specific for TCR Vα8 (B21.14) or Vβ2 (B20.6) elements.

Statistical analyses

Rates of IDDM development in the indicated experimental groups were assessed for statistically significant differences by Kaplan-Meier life table analysis using the Statview 4.5 computer software program (Abacus Concepts, Berkeley, CA).

Results

IDDM is accelerated in NOD mice expressing TCR transgenes from the β cell-autoreactive CD8 T cell clone AI4

We previously isolated from islets of standard 5–6-wk-old NOD female mice, a series of K^d-restricted β cell-autoreactive CD8 T cell clones contributing to the earliest initiative phases of IDDM development (17). Subsequently, rearranged TCR α- and β-chain genes were isolated from one of these T cell clones (AI4) and individually introduced as transgenes directly into NOD mice (designated NOD.AI4α Tg or NOD.AI4β Tg mice). NOD mice carrying both the AI4 TCR α- and β-chain transgenes (designated NOD.AI4$\alpha\beta$ Tg mice) were then selected from intercross progeny. The female incidence of IDDM in these three TCR Tg stocks was then compared with that of transgene negative segregants. Most significantly, when compared with nontransgenic controls, NOD.AI4$\alpha\beta$ Tg mice exhibited a greatly accelerated rate of IDDM development ($p < 0.0001$, Kaplan-Meier life table analysis), with disease first observed as early as 3 wk of age (Fig. 1). The NOD.AI4α Tg line developed IDDM at a slightly faster, but not significantly different rate than nontransgenic controls. Interestingly, expression of the AI4β Tg alone exerted a significant protective effect ($p = 0.0045$) in which both the frequency and rate of IDDM development were inhibited. One possible explanation for these latter two results is that the antigenic specificity of diabetogenic CD8 T cells in NOD mice may be primarily imparted

Table I. *MHC class I-restricted AI4αβ Tg can also be expressed in the CD4 lineage*[a]

Strain	Total TCR Tg$^+$ Cells (% ± SEM)	CD4 T Cells (% ± SEM)	CD8 T Cells (% ± SEM)	Proportion of Total T Cells Expressing TCR Tg[b] (% ± SEM)
NOD.AI4α Tg (n = 5)	29.9 ± 2.2	24.9 ± 2.2	8.1 ± 0.9	91.3 ± 5.6
NOD.AI4β Tg (n = 5)	34.5 ± 1.1	23.5 ± 1.1	11.9 ± 1.1	98.0 ± 2.7
NOD.AI4αβ Tg (n = 6)	21.6 ± 2.1	10.0 ± 1.1	12.3 ± 0.8	96.1 ± 4.4
NOD (n = 3)	0.1 ± 0.04[c]	29.4 ± 0.4	10.0 ± 1.2	0.4 ± 0.08[c]

[a] PBL from the indicated mice were assessed as described in *Materials and Methods* by multicolor FACS analysis for CD4 and CD8 T cell levels and expression of the rearranged AI4 TCR α-chain (Vα8) and/or β-chain (Vβ2) genes.
[b] Calculated for each mouse by dividing total % TCR Tg$^+$ cells by combined % CD4 and CD8 T cells.
[c] Values for standard NOD mice based on proportion of PBL costaining with the TCR Vα8 and Vβ2 Abs used to detect AI4 TCR transgene components.

through expression of particular TCR α-chain rather than β-chain gene rearrangements. Support for this possibility is provided by our previous finding that the CD8 T cells initiating IDDM development in NOD mice utilize a relatively restricted set of TCR α-chains coupled with a diverse array of TCR β-chains (17). However, another mechanism could also account for the relative IDDM resistance of NOD mice expressing the AI4β Tg alone. A greater extent of allelic exclusion is usually observed at the TCR β-chain than α-chain locus (23). As a result, the total T cell repertoire could be less diverse in NOD mice solely carrying the AI4β than AI4α Tg construct, which in turn could limit IDDM development.

The MHC class I-restricted AI4αβ Tg can also be expressed in the CD4 lineage

Due to the process of allelic exclusion, mice carrying rearranged TCR α- and β-chain transgenes predominantly produce the T cell clonotype encoded by the transgenes (24). However, allelic exclusion, particularly within the TCR α-chain is an incomplete process (reviewed in Ref. 23). For this reason, TCR Tg mice usually continue to produce some fraction of T cells that express endogenously derived TCR molecules. Hence, we reasoned that some T cells which fail to express AI4 TCR elements might be exerting IDDM promotive or inhibitory effects in our NOD TCR Tg stocks. Thus, we assessed what types and proportions of T cells in our NOD TCR Tg stocks expressed AI4 TCR elements. In the NOD.AI4α Tg stock, T cells expressing the single Tg TCR α-chain comprised ~30% of the total PBL population (Table I). PBL from this stock consisted of ~25% CD4 and ~8% CD8 T cells. This indicated that although ~91% of the total T cells in NOD.AI4α Tg mice express the Tg TCR α-chain, a significant fraction of these reside within the CD4 rather than the expected CD8 lineage. A similar situation was observed in NOD.AI4β Tg mice (Table I). In the NOD.AI4αβ Tg stock, ~96% of the total T cells expressed the two Tg TCR elements, but half of these still resided within the unexpected CD4 lineage.

TCR transgene-positive CD4 T cells in NOD.AI4αβ Tg mice are selected through coexpression of a second TCR

The data described above suggested the unexpected population of CD4 T cells present in NOD.AI4αβ Tg mice could contribute to their accelerated rate of IDDM development. For this reason, we evaluated the developmental basis of CD4 T cells in the NOD.AI4αβ Tg stock. There have been previous reports that due to incomplete allelic exclusion, TCR Tg mice can develop T cells that express a second endogenously derived TCR (25–27). Thus, although they express the AI4 TCR, we reasoned the selection and functional activities of CD4 T cells in NOD.AI4αβ Tg mice could actually be mediated through their coexpression of a second TCR. Presence of the *scid* mutation blocks the productive rearrangement of germline TCR gene segments (28). The *scid* mutation has already been fixed on the inbred NOD background (20). Thus, we were able to produce through a single outcross-intercross cycle NOD.AI4αβ Tg mice that were also homozygous for the *scid* mutation. FACS analysis revealed a complete absence of CD4 T cells in these NOD-*scid* AI4αβ Tg mice (representative profile in Fig. 2; data summarized in Table II). However, AI4 TCR transgene expressing CD8 T cells remained present. Hence, the CD4 T cells present in standard NOD.AI4αβ Tg mice are not selected through the Tg TCR, but rather a second endogenously derived TCR. To further address this issue, we crossed the AI4αβ Tg constructs onto the previously described stock (14) of NOD mice that were made MHC class I deficient by a functionally inactivated β_2-microglobulin gene (NOD.$\beta 2m^{null}$). Converse to the effect induced by the *scid* mutation, *β2m*-deficient NOD.AI4αβ Tg mice developed CD4, but not CD8 T cells expressing the Tg AI4 TCR (representative profile in Fig. 2; data summarized in Table II). Since the AI4 TCR is restricted to the K^d MHC class I gene product, the above finding also indicated that the CD4 T cells present in standard NOD.AI4αβ Tg mice are actually selected through a second endogenously derived TCR that is MHC class II restricted.

It should also be noted that in standard NOD.AI4αβ Tg mice, as well as in those carrying the *scid* or $\beta 2m^{null}$ mutations, a small population of CD4$^-$CD8$^-$ TCR$^+$ cells could be detected (Fig. 2). A significant fraction of NK T cells have been reported to lack both CD4 and CD8 expression (29). NK T cell development is dependent on expression of the class I-like molecule CD-1. However, the CD4$^-$CD8$^-$ TCR$^+$ cells present in NOD.AI4αβ Tg mice are unlikely to represent NK T cells since they are not eliminated in the presence of the $\beta 2m^{null}$ mutation which blocks CD-1 expression. Instead, we consider it most likely that the small population of CD4$^-$CD8$^-$ TCR$^+$ cells found in NOD.AI4αβ Tg mice develop as a consequence of the transgene-encoded TCR being expressed at an earlier stage of T cell development than is normally the case for endogenously derived TCR molecules. Indeed, a Tg TCR derived from another MHC class I-restricted β cell-autoreactive T cell clone clone of NOD origin (NY8.3) has been shown to be expressed at an early CD4$^-$CD8$^-$ stage of T cell development (30).

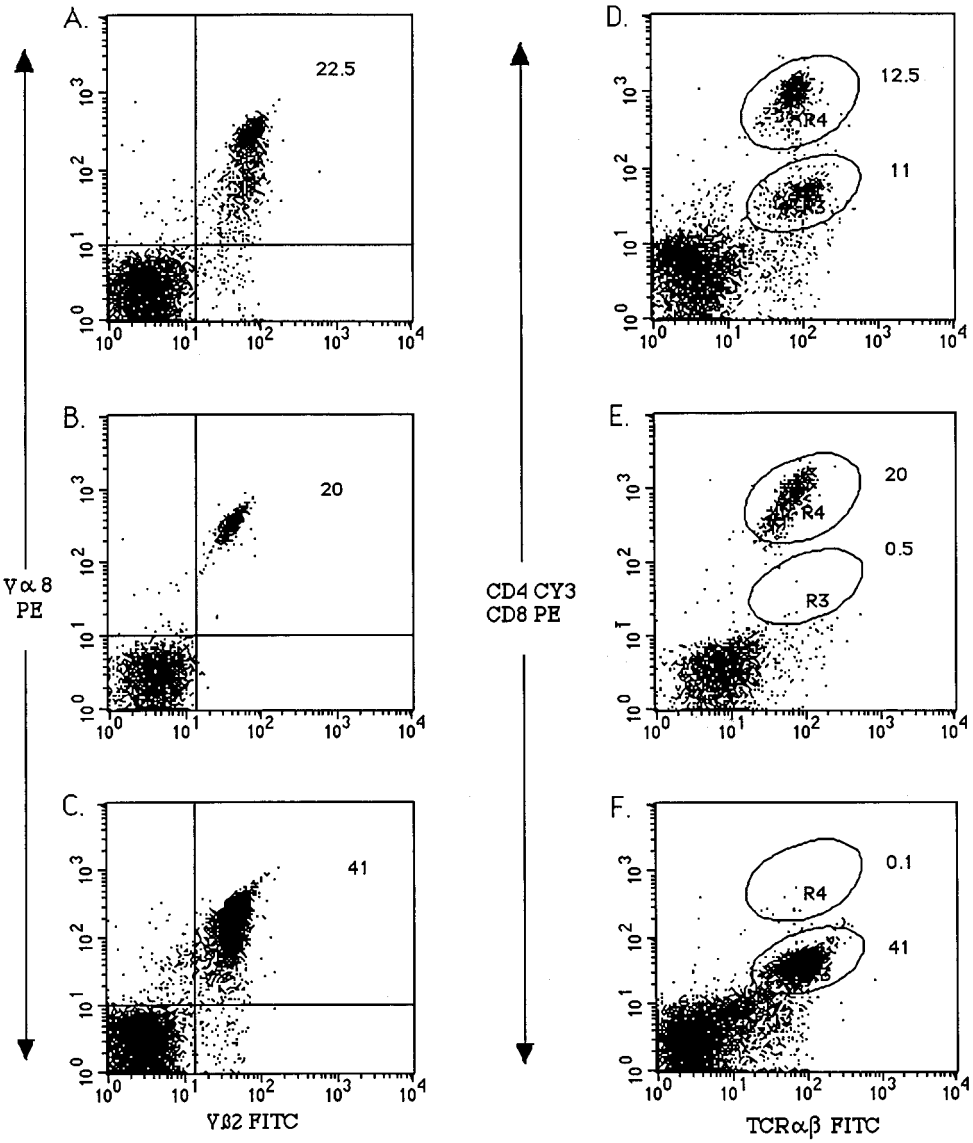

FIGURE 2. Representative FACS profiles of T cell subset alterations in NOD.AI4$\alpha\beta$ Tg mice elicited by the *scid* or $\beta 2m^{null}$ mutations. Total proportions of TCR Tg T cells among PBL in standard NOD.AI4$\alpha\beta$ Tg mice (A) and those homozygous for the *scid* (*B*) or $\beta 2m^{null}$ (C) mutations. *D–F*, Proportions of CD4 (R3 gate) and CD8 T cells (R4 gate) in the same mice. Numbers depict the percentage of cells within the indicated gates.

CD8 T cells expressing the AI4 TCR can independently mediate IDDM development

Perhaps the most striking observation in these studies was that compared with standard NOD mice, IDDM continued to develop at a greatly accelerated rate in the NOD-*scid* AI4$\alpha\beta$ Tg stock ($p < 0.0001$) (Fig. 3). Conversely, while possessing AI4 TCR-positive CD4 T cells, NOD.$\beta 2m^{null}$.AI4$\alpha\beta$ Tg mice were completely IDDM resistant ($p = 0.0002$ vs NOD controls). Histological examination of pancreatic tissue revealed extensive insulitis in NOD-*scid* AI4$\alpha\beta$ Tg mice, whereas islets from NOD.$\beta 2m^{null}$.AI4$\alpha\beta$ Tg mice were completely free of infiltrating leukocytes (data not shown). These collective results indicated that MHC class I-restricted CD8 T cells expressing the AI4 TCR can mediate IDDM

Table II. *Summarized effects of the* scid *or* $\beta 2m^{null}$ *mutations on development of CD4 and CD8 T cells in NOD.AI4$\alpha\beta$ Tg mice*[a]

Strain	Total TCR Tg^+ Cells (% ± SEM)	CD4 T Cells (% ± SEM)	CD8 T Cells (% ± SEM)
NOD-*scid*.AI4$\alpha\beta$ Tg ($n = 3$)	29.2 ± 8.5	0.6 ± 0.3	28.0 ± 8.0
NOD.$\beta 2m^{null}$.AI4$\alpha\beta$ Tg ($n = 5$)	32.8 ± 3.0	35.7 ± 1.8	0.1 ± 0.03

[a] PBL from the indicated mice were assessed as described in *Materials and Methods* by multicolor FACS analysis for CD4 and CD8 T cell levels and expression of the rearranged AI4 TCR α- and β-chain genes.

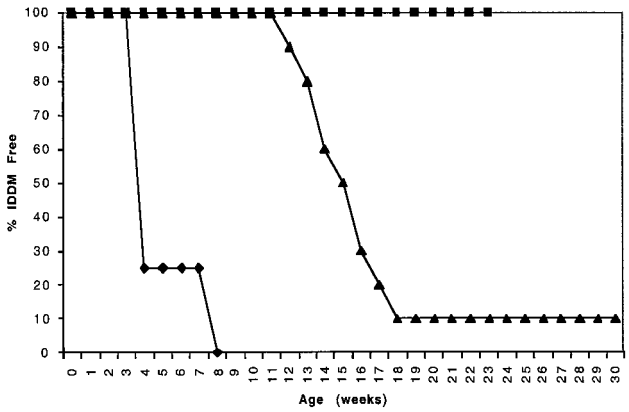

FIGURE 3. IDDM development remains accelerated in NOD.AI4$\alpha\beta$ Tg mice carrying the *scid* mutation, but is completely inhibited by the $\beta 2m^{null}$ mutation. The rate of IDDM development in NOD.AI4$\alpha\beta$ Tg females carrying the *scid* (♦, $n = 4$) or $\beta 2m^{null}$ mutation (■, $n = 5$) was compared with that of standard NOD females (▲, $n = 10$).

development in NOD mice in the complete absence of help provided by MHC class II-restricted CD4 T cells. However, as an additional test of this issue, we crossed the AI4$\alpha\beta$ Tg constructs onto a stock of NOD mice carrying a functionally inactivated CD4 gene ($CD4^{null}$). These NOD.$CD4^{null}$ mice are normally completely IDDM resistant (0/18 females diabetic by 30 wk of age). In contrast, six of eight NOD.$CD4^{null}$ females that expressed the AI4$\alpha\beta$ Tg constructs developed IDDM with a mean age of onset of 7 wk. These results further support the conclusion that MHC class I-restricted AI4 clonotypic T cells can independently mediate the development of autoimmune IDDM in NOD mice.

Discussion

This study demonstrates that when generated in TCR Tg NOD mice, the AI4 CD8 T cell clonotype represents a class of MHC class I-restricted β cell-autoreactive effectors which can progress from a naive to a fully activated state and mediate a high rate of IDDM development in the complete absence of help provided by MHC class II-restricted CD4 T cells. Another MHC class I-restricted β cell-autoreactive T cell clone has been shown to independently transfer IDDM to NOD-*scid* recipients, but these effectors had been previously activated in vitro (31). In contrast, the AI4 T cells which develop in our TCR Tg stock originally arise in a naive state. IDDM development is also accelerated in a stock of NOD mice transgenically expressing the TCR $\alpha\beta$-chain elements from the MHC class I-restricted β cell-autoreactive T cell clone NY8.3 (30). However, the rate and frequency of IDDM development in the NY8.3 TCR Tg stock was greatly reduced when the residual nontransgenic CD4 T cells were eliminated by introduction of a functionally inactivated *Rag-2* allele. In contrast, elimination of residual CD4 T cells by introduction of the *scid* mutation did not impede the greatly accelerated rate of IDDM development observed in the NOD.AI4$\alpha\beta$ Tg stock. Collectively, these results indicate that the β cell-autoreactive CD8 T cells which are essential contributors to IDDM development in NOD mice vary in their helper factor dependence. However, the most important finding in the current study was that some MHC class I-restricted T cell clonotypes, such as AI4, can efficiently mediate autoimmune IDDM development in the complete absence of CD4 T cell helper activities.

Although essential to the process, CD8 T cells isolated from standard NOD mice cannot independently mediate IDDM development (13, 16, 17). This might be due to the fact that in standard NOD mice the frequency of CD8 T cell clonotypes such as AI4 that can independently mediate IDDM development is lower than in a TCR-transgenic situation. Thus, most of the CD8 T cell repertoire that contributes to IDDM development in standard NOD mice is most likely dependent on helper functions provided by CD4 T cells. However, even if their initial expansion in standard NOD mice is aided by helper factors, it is possible that in the preclinical stages of IDDM development, MHC class I-restricted clonotypes with AI4-like characteristics achieve a critical threshold level which could then allow them to independently mediate progression to overt disease. Under this scenario, once AI4-like effectors reach a certain threshold level, IDDM will still develop even if the activities of all MHC class II-restricted β cell-autoreactive T cells are eliminated. This could be of great clinical significance since many envisioned IDDM intervention protocols are targeted at MHC class II-restricted autoreactive T cell responses. Given these concerns, it will be critical to have an understanding of the selection and activation requirements for MHC class I-restricted β cell-autoreactive clonotypes such as AI4 that can independently mediate IDDM development.

Previously, most attention has focused on the way CD4 T cells interact with unusual MHC class II variants to elicit IDDM in both humans and NOD mice. However, the current study, combined with previous findings from Verdaguer et al. (30), clearly indicate that under some circumstances NOD mice can develop IDDM solely through the interaction of CD8 T cells with very common MHC class I gene products that do not contribute to autoimmunity when expressed in other strains. Interestingly, there have been reports that certain common MHC class I alleles are also associated with an increased risk for IDDM development in humans (32–35). Thus, an important future issue will be to determine the mechanisms by which some common MHC class I variants can acquire autoimmune diabetogenic functions when expressed in certain individuals and NOD mice. The most likely scenario is that normally nonpathogenic MHC class I variants can only acquire autoimmune diabetogenic functions through interactions with some of the other susceptibility genes located both within and outside the MHC. The NOD.AI4$\alpha\beta$ Tg stock is likely to be invaluable in determining the mechanisms by which a common MHC class I variant can acquire the aberrant ability to select and functionally activate CD8 T cells capable of independently mediating autoimmune IDDM. For example, it could be outcrossed with IDDM-resistant strains congenic for the NOD $H2^{g7}$ MHC haplotype to map and identify non-MHC genes that may contribute to selection of diabetogenic CD8 T cells.

AI4 represents the first example of a MHC class I-restricted CD8 T cell with an ability to rapidly mediate IDDM development in NOD mice without prior activation in vitro and in the total absence of helper activities. We have found that AI4 T cells do not recognize the K^d-restricted insulin peptide that is the antigenic target of another NOD-derived β cell-autoreactive CD8 clonotype (36). Furthermore, AI4 T cells do not recognize the NRP mimotope of the K^d-restricted β cell autoantigen that is the target of the NY8.3 clone (37). Given the greatly accelerated rate of IDDM development in the NOD.AI4$\alpha\beta$ Tg stock, the MHC class I-restricted antigenic peptide recognized by the AI4 TCR is likely to be of great pathological significance. Thus, its identification could lead to the development of tolerogenic therapies that inhibit IDDM development in NOD mice, which might also be ultimately applicable to humans at risk for this disease.

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