Deficiency in B7-H1 (PD-L1)/PD-1 Coinhibition Triggers Pancreatic β-Cell Destruction by Insulin-Specific, Murine CD8 T-Cells

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OBJECTIVE—RIP-B7.1 mice expressing the costimulator molecule B7.1 (CD80) on pancreatic β-cells are a well established model to characterize preproinsulin-specific CD8 T-cell responses and experimental autoimmune diabetes (EAD). Different immunization strategies could prime preproinsulin-specific CD8 T-cells in wild-type C57BL/6 (B6) mice, but did not induce diabetes. We tested whether altering the B7-H1 (PD-L1) coinhibition on pancreatic β-cells can reveal a diabetogenic potential of preproinsulin-specific CD8 T-cells.

RESEARCH DESIGN AND METHODS—DNA-based immunization and adoptive T-cell transfers were used to characterize the induction of preproinsulin-specific CD8 T-cell responses and EAD in RIP-B7.1, B6, B7-H1−/−, PD-1−/− or bone marrow chimeric mice.

RESULTS—Preproinsulin-specific CD8 T-cells primed in B6 mice revealed their diabetogenic potential after adoptive transfer into congenic RIP-B7.1 hosts. Furthermore, preproinsulin-specific CD8 T-cells primed in anti-B7-H1 antibody-treated B6 mice, or primed in B7-H1−/− or PD-1−/− mice induced EAD. Immunization of bone marrow chimeric mice showed that deficiency of either B7-H1 in pancreatic β-cells or of PD-1 in autoreactive CD8 T-cells induced EAD.

CONCLUSIONS—An imbalance between costimulatory (B7.1) and coinhibitory (B7-H1) signals on pancreatic β-cells can trigger pancreatic β-cell destruction by preproinsulin-specific CD8 T-cells. Hence, regulation of the susceptibility of the β-cells for a preproinsulin-specific CD8 T-cell attack can allow or suppress EAD. Diabetes 59:1966–1973, 2010

Insulin-producing β-cells in the pancreatic islets are destroyed by an immune attack in autoimmune type 1 diabetes. Type 1 diabetes is triggered by a poorly defined breakdown in central or peripheral tolerance that allows activation of diabetogenic T-cells (1,2). Preclinical animal models have elucidated some aspects of the priming and effector phase of a diabetogenic immune response (3,4). Mice develop diabetes either spontaneously in the NOD model (5), or in response to transgene-encoded "neo-self" antigens selectively expressed in pancreatic β-cells (6–8). These studies indicated that priming of self-reactive T-cells and β-cell susceptibility to an autoaggressive T-cell attack are distinct steps in the pathogenesis of the disease.

Costimulating B7/CD28 family molecules provide critical signals for T-cell activation (9,10). RIP-B7.1 mice express the B7.1 costimulator in pancreatic β-cells under rat insulin promoter (RIP) control (11). We have shown that RIP-B7.1 mice develop CD8 T-cell–dependent experimental autoimmune diabetes (EAD) after immunization with preproinsulin-encoding vectors (12–14). Transgene-driven B7.1 expression in pancreatic β-cells thus makes them susceptible to T-cell–mediated immune attack.

Coinhibitory signals generated by "programmed death-1" (PD-1)"programmed death-ligand-1" (B7-H1 or PD-L1) interaction downmodulated T-cell responses and maintain self-tolerance in autoimmune diabetes (15,16). Inducible or constitutive expression of B7-H1 is found in many peripheral tissues, including the β-cells of the pancreatic islets (17,18). Ligation of PD-1 (expressed by activated T-cells) to B7-H1 (expressed by epitope-presenting cells) downmodulates T-cell proliferation and IFNγ production (19). Furthermore, B7-H1 interacts specifically with the costimulatory B7.1 (CD80) molecule upregulated by activated T-cells and inhibits their responses (20). PD-1/B7-H1 interaction facilitates establishment of self-tolerance, thereby partially controlling diabetes development in NOD mice (15,21–23). Selective, transgene-driven overexpression of B7-H1 by pancreatic β-cells can, however, result in EAD, suggesting operation of a costimulatory B7-H1 pathway (24).

We investigated the impact of inhibitory (B7-H1, PD-1) molecules on the pathogenicity of preproinsulin-specific CD8 T-cells. We used RIP-B7.1 mice to characterize the specificity and diabetogenic potential of preproinsulin-specific CD8 T-cell responses. RIP-B7.1 mice were immunized with preproinsulin-encoding vectors, or used as hosts for adoptive T-cell transfers. We further analyzed preproinsulin-specific CD8 T-cell responses and EAD development in C57BL/6 (B6), B7-H1−/− (25), or PD-1−/− knockout mice (26), as well as bone marrow chimeras (using different donor T-cell and host β-cell phenotypes of B7-H1/PD-1).

RESEARCH DESIGN AND METHODS—H-2b C57BL/6 (B6) mice were obtained from Janvier (Le Genets-St-Isle; France), B6.SJL-Ptprc-6 Ptprc-6BoyJ (CD45.1− B6) mice (Jackson #002014), B7-H1−/− mice (25), PD-1−/− mice (26), and RIP-B7.1 mice (11) were bred and...
kept under standard pathogen-free conditions in the animal colony of Ulm University (Ulm, Germany). All studies were conducted after Institutional Board approval in accordance with the Federal German Animal Protection Law.

Immunization of mice. Mice were immunized intramuscularly into the tibialis anterior muscle or injected intravenously with 30 μg of the indicated peptides in the presence of brefeldin A (0.5 μg/ml) (cat. no. 15870; Sigma, Taufkirchen, Germany). Cells were harvested, surface stained with APC-conjugated anti-CD8 antibody (cat no. 17–0081–83, BD Biosciences, Heidelberg, Germany), fixed with 2% paraformaldehyde, resuspended in permeabilization buffer (HBSS, 0.5% BSA, 0.5% saponin, 0.05% sodium azide), and stained with fluorescein isothiocyanate-conjugated anti-IFNγ antibody (cat. no.554411; BD Biosciences, Heidelberg, Germany). Frequencies of IFNγ+ CD8 T-cells were determined by flow cytometry (FCM).

RESULTS

EAD induced in the RIP-B7.1 model. A single injection of the pCI/preproinsulin DNA encoding murine preproinsulin efficiently induced hyperglycemia in RIP-B7.1 mice, but not in wild-type B6 mice (Fig. 1A) (13,14). pCI/preproinsulin DNA was electroporated into the tibialis anterior muscle or injected intravenously into the RIP-B7.1 model mice. These mice were monitored for 20 weeks post immunization. Cumulative diabetes incidences (%) were determined (Fig. 1B). Diabetic RIP-B7.1 mice showed a 100% incidence of diabetes, whereas the wild-type B6 mice did not develop diabetes (Fig. 1B). The mean percentage of CD8 T-cells in the pancreatic CD8 T-cell population (obtained from two independent experiments) were shown (Fig. 1C). Determination of specific CD8 T-cell frequencies. Pancreatic cells were prepared from pCI/preproinsulin (group 1) or pCI/preproinsulin N110A (group 2) immunized, diabetic RIP-B7.1 mice, or control pCI-immunized, healthy (group 3) RIP-B7.1 mice and restimulated ex vivo with the K b/A12-N21A or K b/OVA257–264 peptides, and specific IFNγ+ CD8 T-cell levels were determined by flow cytometry. The mean percentage of IFNγ+ CD8 T-cells in the pancreatic CD8 T-cell population (+ SD) of a representative experiment (n = three mice per group) are shown. pCl/ppins, pCl/preproinsulin.

FIG. 1. The RIP-B7.1 diabetes model. A: RIP-B7.1 or C57BL/6 (B6) mice were immunized with pCI/preproinsulin (●, n = 8) or the noncoding pCl (○, n = 8). At indicated times after immunization cumulative diabetes incidences (%) were determined. B: RIP-B7.1 mice were immunized with pCl/preproinsulin DNA. At 3 weeks after immunization, we selected three mice that had developed an early stage of EAD (with blood glucose levels between 300–440 mg/dl). These mice were injected twice (at days 21 and 23) with 200 μg mAb YTS-169 (anti-CD8). The glucose level (●, mg/dl) and CD8 T-cell numbers in the blood (○, CD8 T-cells number of nontreated mice were set for 100%) were determined at indicated time points after immunization. The injections of pCl/preproinsulin DNA and anti-CD8 mAb are indicated (arrows). C: RIP-B7.1 mice (n = 10) were immunized with pCl/preproinsulin DNA. Pancreatic CD8 T-cells derived from diabetic mice (blood glucose level >400 mg/dl) were pooled and restimulated ex vivo with a preproinsulin-specific peptide library (i.e., 10mers with two amino acids offset), and frequencies of IFNγ+ CD8 T-cells were determined by flow cytometry. The mean percentage of IFNγ+ CD8 T-cells in the pancreatic CD8 T-cell population (obtained from two independent experiments) are shown. D: Pancreatic cells were prepared from pCl/preproinsulin (group 1) or pCl/preproinsulin N110A (group 2) immunized, diabetic RIP-B7.1 mice, or control pCl-immunized, healthy (group 3) RIP-B7.1 mice and restimulated ex vivo with the K b/A12-N21A or K b/OVA257–264 peptides, and specific IFNγ+ CD8 T-cell levels were determined by flow cytometry. The mean percentage of IFNγ+ CD8 T-cells in the pancreatic CD8 T-cell population (+ SD) of a representative experiment (n = three mice per group) are shown. pCl/ppins, pCl/preproinsulin.
preproinsulin-immunized RIP-B7.1 mice with early EAD (blood glucose levels of 300–440 mg/dl) were treated 3 weeks after immunization with anti-CD8 antibody (Fig. 1B). This antibody treatment efficiently depleted CD8 T-cells within 2–3 days and transiently cured EAD (Fig. 1B). CD8 T-cell levels were restored 3–4 weeks after the anti-CD8 antibody treatment was discontinued, and EAD reappeared concomitant with the re-emerging CD8 T-cells (Fig. 1B). Anti-CD4 antibody treatment did not inhibit diabetes progression (data not shown). The effector phase of EAD thus depends on diabetogenic CD8 T-cells.

Characterization of diabetogenic CD8 T-cells. EAD development in preproinsulin-immunized RIP-B7.1 mice was accompanied by increasing infiltrations of CD8 T-cells into the pancreatic target tissue (13,14). CD8 T-cells isolated from immunized, diabetic RIP-B7.1 mice specifically recognized the immunodominant K\(^{\alpha}\)-restricted A\(_{12-21}\) (i.e., preproinsulin\(_{101-110}\)) epitope of preproinsulin (Fig. 1C) (13,27). Ex vivo stimulation of preproinsulin-primed, pancreas-infiltrating CD8 T-cells with the antigen A\(_{12-21}\) peptide, but not with all other peptides of a preproinsulin-specific library, revealed a CD8 T-cell population with specifically inducible IFN\(\gamma\) expression (Fig. 1C). In the course of EAD, we also found a significant influx of other lymphoid cells (e.g., CD4 T-cells and B cells) of unknown specificities into the pancreata (data not shown) (14). It is under investigation whether these bystander cells contribute to the diabetogenic immune response (5,28).

We usually detected 4–8 \(\times\) 10^3 CD8 T-cells per pancreas in preproinsulin-immunized, diabetic RIP-B7.1 mice (with blood glucose levels of >400 mg/dl). The frequency of A\(_{12-21}\)-specific IFN\(\gamma^{+}\) CD8 T-cells in these pancreatic CD8 T-cell populations was low (0.5–1.5%) (Fig. 1C). We identified an epitope variant (A\(_{12-N21A}\)) with an alanine (A) exchange for the COOH-terminal asparagine (N) at position A\(_{21}\). This variant facilitated in vitro detection of primed CD8 T-cells (14). RIP-B7.1 mice immunized with either pCI/preproinsulin or the pCI/preproinsulin\(_{N110A}\) variant (encoding preproinsulin with the mutant A\(_{12-N21A}\) epitope) developed a similar EAD (data not shown). Pancreas-infiltrating IFN\(\gamma^{+}\) CD8 T-cells expanded in vitro more efficiently after stimulation with the mutant A\(_{12-N21A}\) than the A\(_{12-21}\) peptide (Fig. 1D). Similarly, a monospecific A\(_{12-21}\)-encoding vector induced EAD in RIP-B7.1 mice (14), but pancreas-infiltrating CD8 T-cells preferentially expanded in vitro with the mutant A\(_{12-N21A}\) peptide (data not shown). Hence, the alanine at position A\(_{21}\) of the mutant epitope apparently modulates the specific steric structure of the peptide (29) and improves its major histocompatibility complex class I-presentation properties in vitro. We used the variant epitope to detect preproinsulin-specific CD8 T-cell responses.

The effector phase of diabetogenic, preproinsulin-specific CD8 T-cell responses is blocked in C57BL/6 (B6) mice. Preproinsulin-specific immunization efficiently induced EAD in RIP-B7.1 mice, but not in B6 mice (Fig. 1A). However, low numbers of CD8 T-cells were reproducibly found in the periphery of some (\(<2\%\)) islets in immunized B6 mice (Fig. 2A). These CD8 T-cell populations were not found either in nonimmunized B6 (Fig. 2A) or nonimmunized RIP-B7.1 mice (13,14), suggesting that immunization had induced preproinsulin-specific CD8 T-cells in B6 mice.

We used adoptive transfer experiments to test whether functional preproinsulin-specific CD8 T-cells are primed in B6 mice. B6 mice were immunized with pCI/preproinsulin\(_{N110A}\). Their splenic CD8 T-cells isolated 14 days after immunization were adoptively transferred into RIP-B7.1 mice (30). RIP-B7.1 hosts developed EAD after transfer with primed (Fig. 2B, group 2), but not with nonprimed (Fig. 2B, group 1), CD8 T-cells. Preproinsulin-specific CD8 T-cells accumulated in the pancreata of transplanted and diabetic hosts (Fig. 2C, group 2). IFN\(\gamma^{+}\) is critical for inducing EAD in RIP-B7.1 hosts because adoptively transferred CD8 T-cells from immunized IFN\(\gamma^{+}\)-mice did not induce EAD (Fig. 2B, group 3). Similarly, immunization of RIP-B7.1/\(\alpha\)-IFN\(\gamma^{+}\)-mice with preproinsulin also did not induce EAD (13).

B7-H1 expression by pancreatic \(\beta\)-cells controls the diabetogenic CD8 T-cell response. We tested whether EAD can be triggered in preproinsulin-immune B6 mice by blocking B7-H1-mediated co-inhibition. B6 mice injected with either pCI (Fig. 3A) or pCI/preproinsulin\(_{N110A}\) (Fig. 3B and C) were treated at days 12 and 15 after immunization, either with blocking B7-H1 antibody (31) (Fig. 3A and B) or an isotype control antibody (Fig. 3C). PC1/preproinsulin\(_{N110A}\)-immunized B6 mice rapidly developed hyperglycemia after injection of the anti-B7-H1, but not control antibody (Fig. 3B and C). Control mice (injected with pCI and treated with anti-B7-H1 antibody) did not develop EAD (Fig. 3A).

Within 6–9 days after anti-B7-H1 antibody injection, mice developed either severe hyperglycemia (Fig. 3B, group 1) or moderate and transient hyperglycemia (Fig. 3B, groups 2a/b). Severe disease correlated with an influx of high levels of A\(_{12-N21A}\)-specific IFN\(\gamma^{+}\) CD8 T-cells into the islets and an almost complete loss of insulin-producing islet \(\beta\)-cells (Fig. 3D and E; group 1). In mice with transient diabetes, only low numbers of CD8 T-cells were detectable in the islets during the hyperglycemic stage, and insulin expression was reduced, but still intact (Fig. 3D and E; group 2a). After recovery to normoglycemia, CD8 T-cells were no longer detectable in the islets (Fig. 3D and E; group 2b). Pancreatic \(\beta\)-cells that lose B7-H1 co-inhibition are hence susceptible (at least transiently) to attack by preproinsulin-specific CD8 T-cells.

We used B7-H1\(^{-/-}\) mice (25) to confirm that EAD induction by preproinsulin depends on B7-H1. A single injection of pCI/preproinsulin\(_{N110A}\)-induced hyperglycemia in B7-H1\(^{-/-}\) mice. EAD developed in both male and female B7-H1\(^{-/-}\) mice with a median onset of 3–5 weeks after immunization and a cumulative diabetes incidence of 80% by week 5 (Fig. 4A, data not shown). B7-H1\(^{-/-}\) mice did not develop spontaneous EAD after injection with the noncoding pCI vector (Fig. 4A). Using the preproinsulin-specific peptide library described above (see Fig. 1C), we detected only A\(_{12-21}\)/A\(_{12-N21A}\)-specific IFN\(\gamma^{+}\) CD8 T-cells in the pancreata of immunized and diabetic B7-H1\(^{-/-}\) mice (data not shown). Thus, CD8 T-cells with this specificity play a prominent role in the destructive autoimmune response in these mice.

We used adoptive cell transfers to exclude that B7-H1-deficiency in CD8 T-cells is critical for EAD development in B7-H1\(^{-/-}\) mice. RIP-B7.1 and B7-H1\(^{-/-}\) mice were immunized with pCI/preproinsulin\(_{N110A}\). We adoptively transferred 3 \(\times\) 10^6 CD8 T-cells (derived from spleens of early diabetic mice) into RIP-B7.1 hosts (Fig. 4B). RIP-B7.1 hosts reconstituted with primed, RIP-B7.1- or B7-H1\(^{-/-}\)-derived CD8 T-cells induced EAD with a similar efficacy (Fig. 4B). Transfer of nonprimed CD8 T-cells from healthy RIP-B7.1 or B7-H1\(^{-/-}\) donors did not induce EAD (data not
shown). Thus, B7-H1-deficiency in preproinsulin-specific CD8 T-cells did not alter their diabetogenic potential. In contrast, transfer of identical RIP-B7.1-derived CD8 T-cell preparations into B7-H1−/− hosts inefficiently induced late EAD (Fig. 4C). Only 1 of 8 B7-H1−/− hosts developed EAD at 18 weeks after transfer (Fig. 4C). The efficacy of EAD could be increased in adoptively transferred B7-H1-deficient hosts if the stimulatory B7.1 molecule is coexpressed in pancreatic β-cells of RIP-B7.1+/−/B7-H1−/− mice (Fig. 4C).

**B7-H1/PD-1 coinhibition controls diabetogenic CD8 T-cells.** We used PD-1−/− knockout mice (26) to test whether induction of preproinsulin-specific EAD depends on the coinhibitory PD-1/B7-H1 interaction (19). A single injection of the pCI/preproinsulinN110A (but not of pCI vector) into PD-1−/− mice induced A12-N21A-specific IFNγ+ CD8 T-cell responses and EAD (Fig. 4D; data not shown), suggesting that B7-H1/PD-1 coinhibition is critical to induce EAD in preproinsulin-immune mice. To confirm this, we generated bone marrow chimeras. To distinguish between donor- and host-derived T-cells in bone marrow (BM) chimeras, we used wild-type CD45.1+ B6 mice in this set of experiments. BM cells from CD45.1+ donor mice (B7-H1+ PD-1+) were transferred into lethally irradiated, congenic B7-H1−/− or PD-1−/− (CD45.2+), and BM cells from B7-H1−/− or PD-1−/− donor mice were transferred into wild-type CD45.1+ hosts (Table 1). At 6 to 7 weeks after transplantation, chimeric mice contained >90% of donor T-cells (data not shown). Chimeric mice were immunized 7 weeks after transplantation with pCI/preproinsulinN110A (or control pCI). As is evident from Table 1, wild-type/B7-H1−/− (group 1) and PD-1−/−/wild-type chimeras (group 7), but not wild-type/PD-1−/− (group 3) and B7-H1−/−/wild-type chimeras (group 5), developed EAD after immunization with pCI/preproinsulinN110A. EAD manifestation in groups 1 and 7 correlated with an influx of A12-N21A-specific IFNγ+ CD8 T-cells into the target tissue (data not shown). Hence, either the selective deficiency of B7-H1 on target cells (group 1) or the deficiency of PD-1 on T-cells (group 7) triggered CD8 T-cell–mediated EAD. Binding of PD-1 expressed by activated CD8 T-cells to B7-H1 expressed by pancreatic β-cells thus seems to control the diabetogenic immune response.

**DISCUSSION**

There is increasing evidence from patients with type 1 diabetes that autoreactive CD8 T-cells specific for preproinsulin are involved in β-cell destruction (32–38). We established mouse models to study the pathogenic cross-talk between preproinsulin-specific CD8 T-cells and preproinsulin-expressing β-cells. RIP-B7.1 tg and two well-defined (B7-H1−/−, PD-1−/−) mouse lines allowed us to identify critical checkpoints for the control of diabetogenic, preproinsulin-specific CD8 T-cells: 1) the costimulator molecule B7.1 (CD80) expressed on β-cells (RIP-B7.1 mice); 2) the coinhibitor B7-H1 (PD-L1) expressed by
β-cells (B7-H1−/− mice); or 3) the coinhibitor PD-1 molecule expressed by CD8 T effector cells (PD-1−/− mice).

Transgene-driven B7.1 (CD80) expression in the pancreatic β-cells of RIP-B7.1 mice makes them susceptible to autoreactive CD8 T-cell attack. The nonphysiologic, co-stimulatory B7.1/CD28 interaction in RIP-B7.1 mice (9,10) may allow efficient effector function delivery by autoreactive CD8 T-cells. CD28-deficient RIP-B7.1−/−/CD28−/− mice did not develop EAD after immunization with preproinsulin (data not shown). There is thus strong evidence that the interaction of B7.1 on the surface of β-cells with the costimulator molecule CD28 on T-cells is an essential component of T-cell–mediated EAD in RIP-B7.1 mice. Preproinsulin-specific immunization induced EAD in almost all RIP-B7.1 mice with a strikingly similar time course and histopathology. Expression of B7.1 in islet β-cells facilitated diabetes development by adaptively transferred preproinsulin-specific CD8 T-cells (Fig. 4C) (30). The RIP-B7.1 diabetes model is thus well suited to study distinct events in the priming and effector phase of preproinsulin-specific CD8 T-cells. For example, we previously showed that expression and processing of preproinsulin antigens in the endoplasmic reticulum favor priming of autoreactive CD8 T-cells (14). Thus, direct loading of the A12–21 epitope on newly synthesized Kb-molecules in the endoplasmic reticulum may be an essential step for enabling presentation of this epitope (14). We currently establish a HLA-A*0201/RIP-B7.1 mouse model to define the diabetogenic potential of HLA-A*0201-restricted preproinsulin epitopes associated with human type 1 diabetes (35–37). Furthermore, RIP-B7.1 mice are a useful tool to identify novel β-cell antigens that are targets for CD8 T-cell–triggered diabetes (4).

We consider the key observation of this report to be the de novo induction of preproinsulin-specific CD8 T-cells (and EAD) in mice and their control by B7-H1/PD-1 interaction. It has been shown that PD-1 and its ligands, B7-H1 (PD-L1) and PD-L2, deliver inhibitory signals that...
regulate the balance between T-cell activation, tolerance, and immunopathology (19,39). B7-H1 (PD-L1) is expressed on antigen-presenting cells as well as effector CD8 T-cells, whereas PD-1 is expressed on T-cells. Using bone marrow chimeric mice, we confirmed that the deficiency of B7-H1 on target cells or PD-1 on T-cells was essential to trigger preproinsulin-specific, CD8 T-cell–mediated EAD by DNA-based immunization (Table 1). Binding of PD-1 on activated T-cells to B7-H1 expressed by pancreatic β-cells may hence downmodulate the diabetogenic potential of preproinsulin-specific CD8 T-cells. We further showed that B7-H1-deficiency in preproinsulin-specific CD8 T-cells did not alter their diabetogenic potential (Fig. 4B). B7-H1−/− mice inefficiently developed EAD after adoptive transfer of preproinsulin-specific CD8 T-cells, but coexpression of the stimulatory B7.1 molecule in B7-H1-deficient pancreatic β-cells (RIP-B7.1+/B7-H1−/− mice) significantly accelerated disease induction (Fig. 4C). Transgene-driven B7.1 costimulation in pancreatic β-cells was thus more potent than the loss of B7-H1 co-inhibition in promoting the pathogenic immune response of adoptively transferred preproinsulin-specific CD8 T-cells. In contrast, immunization of B7-H1−/− and RIP-B7.1 mice with preproinsulin-encoding DNA induced EAD with similar efficacies and kinetics (see Figs. 1A, 4A). We assume that additional factors (e.g., professional antigen-presenting dendritic cells or cells from the innate immune system) (40) are triggered by DNA-based immunization that facilitate expansion of preproinsulin-specific CD8 T-cells and/or maintain their diabetogenic potential in B7-H1−/− mice.

The B6 diabetes model described in this study is attractive to characterize distinct events in the regulation of β-cell susceptibility to manifest or control preproinsulin-specific, CD8 T-cell–mediated EAD. B6 is not a privileged strain for type 1 diabetes studies, but it was unexpectedly easy to prime preproinsulin-specific CD8 T-cells in male and female B6 mice. CD8 T-cells revealed their diabetogenic potential after adoptive transfer into congenic RIP-B7.1 hosts or after conditioning the pancreatic target tissue by antibody-mediated blockade of B7-H1 co-inhibition (Figs. 2 and 3). Preproinsulin-specific CD8 T-cells in immunized B6 mice thus have a diabetogenic potential, but pancreatic β-cells are protected from immune attack by these cells. Similarly, in the lymphocytic choriomeningitis

FIG. 4. Preproinsulin-specific EAD development in B7−H1−/− and PD-1−/− mice. A: B7-H1−/− mice were immunized with pCI/preproinsulinN110A (●, n = 5), or the noncoding pCI (○, n = 8), and cumulative diabetes incidences (%) were determined. B: RIP-B7.1 (●) and B7-H1−/− (○) mice were immunized with pCI/preproinsulinN110A. Spleens were removed from early diabetic mice and 3 × 10^6 CD8 T-cells were transferred intravenously into sublethally irradiated B7−H1−/− (○, n = 8) or RIP-B7.1+/B7-H1−/− (●, n = 4) hosts. C: PD-1−/− mice were immunized with pCI/preproinsulinN110A (●, n = 12), or the noncoding pCI (○, n = 12), and cumulative diabetes incidences (%) were determined. pCI/ppinsN110A, pCI/preproinsulinN110A.

TABLE 1
Preproinsulin-specific EAD induction in bone marrow chimeras

| Group | Bone marrow chimera | Donor | Host | Immunization | EAD |
|-------|---------------------|-------|------|--------------|-----|
| 1     | wt/B7-H1−/−         | CD45.1+ wt | B7-H1−/− | pCI/ppinsN110A | 4/4 |
| 2     | wt/B7-H1−/−         | CD45.1+ wt | B7-H1−/− | pCI | 0/3 |
| 3     | wt/PD-1−/−          | CD45.1+ wt | PD-1−/− | pCI/ppinsN110A | 0/4 |
| 4     | wt/PD-1−/−          | CD45.1+ wt | PD-1−/− | pCI | 0/3 |
| 5     | B7-H1−/−/wt         | B7-H1−/− | CD45.1+ wt | pCI/ppinsN110A | 0/4 |
| 6     | B7-H1−/−/wt         | B7-H1−/− | CD45.1+ wt | pCI | 0/2 |
| 7     | PD-1−/−/wt          | PD-1−/− | CD45.1+ wt | pCI/ppinsN110A | 2/4 |
| 8     | PD-1−/−/wt          | PD-1−/− | CD45.1+ wt | pCI | 0/4 |

Bone marrow chimeras were generated by injecting wild-type CD45.1+ bone marrow cells intravenously into lethally irradiated (950 rad) B7-H1−/− (CD45.2+) or PD-1−/− (CD45.2+) hosts, or by injecting B7-H1−/− or PD-1−/− bone marrow cells into wild-type CD45.1+ hosts. Mice were injected 7 weeks after transplantation with either the pCI/ppinsN110A, or control pCI plasmid DNA. Blood glucose levels were determined, and the number of diabetic mice per group is indicated. wt, wild-type; ppinsN110A, preproinsulinN110A.
gitsu virus diabetes model, the pancreatic target tissue must be exposed to stimulatory signals from the innate immune system to become susceptible to the destructive CDS T-cell attack (8). The B6 model is a good example for translational medicine since our data illustrated the central role of the β-cell as a gatekeeper in the preproinsulin-specific insulitic process. Beta cells per se prevent the deleterious cross-talk with preproinsulin-specific CDS T-cells. Changes in the β-cell milieu—e.g., by antibody treatment (Fig. 3), by interferon (8), or by viral infections (41)—can favor the susceptibility of β-cells for the CDS T-cell-mediated immune attack. Further manipulations of the pancreatic β-cells or distinct arms of the immune system by specific drugs (42) or by using different mouse strains with defects in specific cell types or immune mediators may define conditions that inactivate (tolerize/anergize) autoreactive CDS T-cells. “Translation” of these approaches to human type 1 diabetes (3,41–43) could be helpful to design prophylactic vaccines.

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T.R., H.B., C.S., and A.S. researched data and contributed to discussion. B.O.B. contributed to discussion and reviewed/edit the manuscript. L.C. reviewed/edit the manuscript. J.R. contributed to discussion and wrote the manuscript. R.S. researched data and wrote the manuscript.

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