Selective delivery of β cell antigen to dendritic cells *in vivo* leads to deletion and tolerance of autoreactive CD8$^+$ T cells in NOD mice

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Type 1 diabetes (T1D) is an autoimmune disease resulting from defects in central and peripheral tolerance and characterized by T cell-mediated destruction of islet β cells. Cytotoxic CD8$^+$ T cells, reactive to β cell antigens, are required for T1D development in the NOD mouse model of the disease, and CD8$^+$ T cells specific for β cell antigens can be detected in the peripheral blood of T1D patients. It has been evident that in nonautoimmune-prone mice, dendritic cells (DCs) present model antigens in a tolerogenic manner in the steady state, e.g., in the absence of infection, and cause T cells to proliferate initially but then to be deleted or rendered unresponsive. However, this fundamental concept has not been evaluated in the setting of a spontaneous autoimmune disease. To do so, we delivered a mimotope peptide, recognized by the diabetogenic CD8$^+$ T cell clone AI4, to DCs in NOD mice via the endocytic receptor DEC-205. Proliferation of transferred antigen-specific T cells was initially observed, but this was followed by deletion. Tolerance was achieved because rechallenge of mice with the mimotope peptide in adjuvant did not induce an immune response. Thus, targeting of DCs with β cell antigens leads to deletion of autoreactive CD8$^+$ T cells even in the context of ongoing autoimmunity in NOD mice with known tolerance defects. Our results provide support for the development of DC targeting of self antigens for treatment of chronic T cell-mediated autoimmune diseases.

autoimmune disease | type 1 diabetes

Type 1 diabetes (T1D) is an autoimmune disease characterized by an inability to establish and maintain tolerance to β cell antigens. In diabetes-prone individuals, autoreactive T cells respond to pancreatic islet β cell antigens in conjunction with costimulatory signals, promoting initial T cell activation resulting in selective expansion, differentiation, islet invasion, and ultimately destruction of β cells (1). Clinically, the end result is the inability of the affected individual to produce the insulin required to properly regulate glucose metabolism. NOD mice provide a model system for T1D that shares many of the characteristics of the human disease (2). Multiple lines of investigation have demonstrated the importance of CD8$^+$ T cells in the pathogenesis of T1D in NOD mice (3). This is consistent with the detection of islet antigen-specific CD8$^+$ T cells in the peripheral blood of T1D patients (4). Using CD8$^+$ T cells derived from the islets of NOD mice, a limited number of β cell antigens recognized by islet-infiltrating T cells have been identified (4). Lack of availability of a therapy for T1D other than insulin administration inspires the usage of β cell antigens and epitopes targeted by T cells in T1D to develop antigen-based tolerogenic strategies.

As reviewed (5), dendritic cells (DCs) in the steady state, e.g., in the absence of infection, present antigens in a tolerogenic manner and cause naive CD8$^+$ T cells to proliferate initially but then to be deleted or rendered unresponsive. The pathway by which DCs acquire exogenous antigens and process them directly for display on class I MHC molecules is known as cross-presentation. DEC-205 (CD205), an endocytic receptor with 10 membrane-external contiguous C-type lectin domains (6), is expressed at high levels on DCs in the T cell areas of lymphoid organs and is one of the DC surface receptors that facilitates this process (7). Like naive T cells, antigen-experienced, including memory, CD8$^+$ T cells are also subject to peripheral tolerance in response to cross-presented antigen (8). Processing and presentation of self antigens by steady-state DCs are now thought to be major components of the establishment of tolerance in the periphery. Interestingly, autoimmune-prone NOD mice have several reported defects in DC populations, including a deficiency in the number of DEC-205$^+$ DCs (9–12).

Antigens can be experimentally targeted to DCs *in vitro* or *in vivo* via the DEC-205 receptor by introducing antigen into an antibody to the receptor (13–16), and this increases the efficiency of presentation of antigens on both MHC class I and class II products *in vivo* (13, 15, 17, 18). Selective presentation in the steady state of a foreign antigen by DCs *in vivo* leads to deletion of reactive CD8$^+$ T cells and the establishment of tolerance in nonautoimmune-prone C57BL/6 mice (13, 19). Selective DC-based presentation of a natural self antigen to CD8$^+$ T cells in the setting of a spontaneous autoimmune disease has yet to be explored but is of considerable biological and clinical interest. Here, we have used targeted delivery of a mimotope of a β cell peptide to DEC-205 in NOD mice and have found that CD8$^+$ T cell tolerance could be achieved even in the face of ongoing autoimmunity and in mice with multiple reported tolerance defects (20–23) and DC abnormalities (9–12).

Results

Preparation and Characterization of a Hybrid Antibody to Be Used for the Tolerization of β Cell-Autoreactive CD8$^+$ T Cells. AI4 is a pathogenic CD8$^+$ T cell clone, isolated from the islets of a 5-wk-old female NOD mouse capable of mediating T1D in the absence of CD4$^+$ T cell help (24). AI4 T cells recognize the

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Conflict of interest statement: R.M.S. advised Celldex, which is developing anti-DEC-205 antibodies for use in humans.

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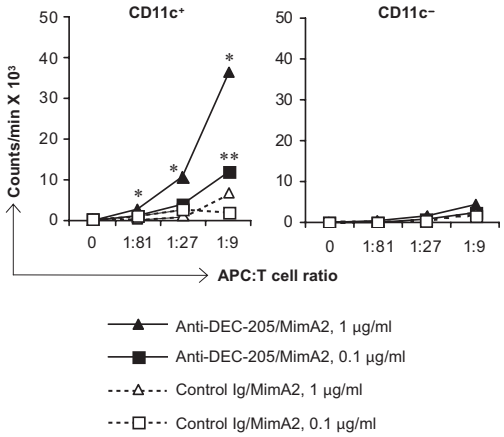

Fig. 1. Presentation of peptides derived from anti-DEC-205/MimA2 is restricted to CD11c$^+$ cells *in vitro*. Splenic DCs (CD11c$^+$ cells) (*Left*) or CD11c$^-$ cells (*Right*) were isolated from NOD mice and incubated overnight with the indicated antibodies. CD8$^+$ T cells were purified from the spleens of NOD.*AI4*$\alpha\beta$Tg mice, and 5×10^4 AI4 T cells were incubated at the indicated ratios with APCs in 96-well plates. After 3 d of coculture, proliferation was monitored by [^{3}H]thymidine incorporation. Data shown are representative of at least two similar experiments. Significant differences between corresponding anti-DEC-205 and control Ig values are indicated: *, $P < 0.05$; **, $P < 0.01$.

superagonist peptide MimA2 in the context of the class I MHC molecule H-2D^b (25). We constructed a hybrid anti-DEC-205 antibody linked with MimA2 [designated anti-DEC-205/MimA2; see *Materials and Methods* and supporting information (SI) Fig. S1*A*] to develop a strategy to manipulate β cell-autoreactive CD8$^+$ T cells in the context of a spontaneous autoimmune disease. The hybrid and unmodified antibodies bound similarly to a stably transfected CHO cell line expressing DEC-205 on its surface (Fig. S1*B*).

To determine whether antigen delivered by anti-DEC-205 can be processed for presentation to CD8$^+$ T cells, we purified CD11c$^+$ DCs and CD11c$^-$ cells from the spleens of NOD mice and incubated them overnight with anti-DEC-205/MimA2 or a control Ig, also fused to MimA2 but unable to bind DEC-205 (control Ig/MimA2). The antigen-presenting cells (APCs) were then cocultured for 2 d with MimA2-specific CD8$^+$ AI4 T cells purified from NOD.*AI4*$\alpha\beta$Tg mice, and T cell proliferation was monitored by [^{3}H]thymidine incorporation. The DCs treated with anti-DEC-205/MimA2 selectively stimulated AI4 T cell proliferation, whereas those treated with control Ig/MimA2 were less active (Fig. 1). The hybrid anti-DEC-205 antibody therefore enhances cross-presentation of the diabetogenic MimA2 epitope by DCs.

Anti-DEC-205 Antibody Delivers a Superagonist Mimotope of a β Cell Antigen for Presentation on Class I MHC *in Vivo*. To determine whether anti-DEC-205/MimA2 could be used for *in vivo* delivery of the MimA2 peptide, we transferred carboxyfluorescein diacetate succinimidyl ester (CFSE)-labeled *Thy1*^{b} AI4 T cells to NOD.NON-*Thy1*^{a} recipients and then treated these recipients with anti-DEC-205/MimA2 or control Ig/MimA2. After 3 d, peripheral lymph nodes, pancreatic lymph nodes, and spleens were harvested and stained with anti-Thy1.2 and -CD8 to identify the transferred cells for examination by flow cytometry (Fig. 2*A*). Extensive proliferation was observed in the peripheral lymph nodes and spleens of anti-DEC-205/MimA2-treated animals (with 64 ± 19% and 65 ± 28% of cells showing CFSE dilution, respectively; $n = 2$), but far less in animals treated with control Ig/MimA2 (26 ± 15% and 24 ± 13%) or PBS (16 ± 9.2% and 13 ± 3.5%). However, all mice showed T cell proliferation

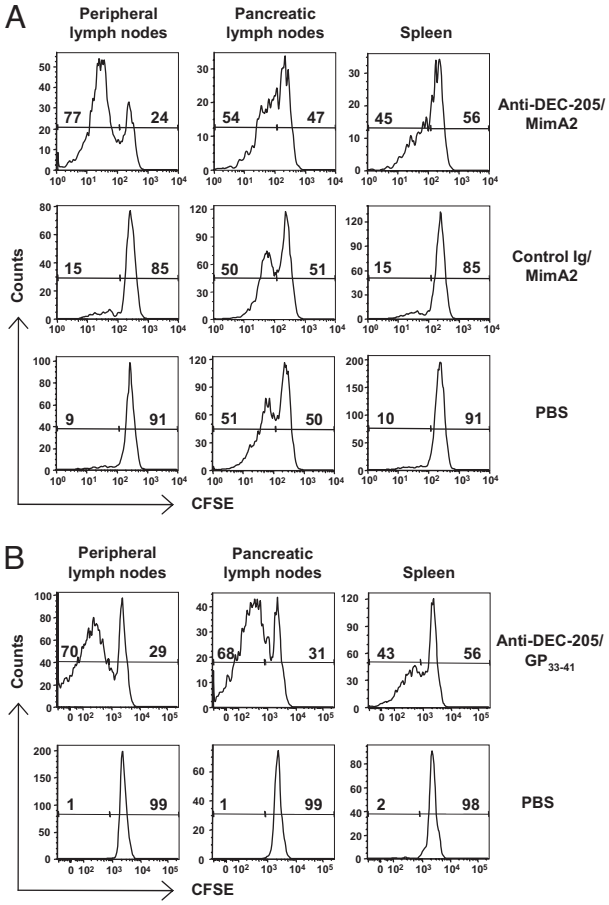

Fig. 2. *In vivo* targeting of peptide-linked anti-DEC-205 results in similar proliferation of CD8$^+$ T cells specific for a self or foreign peptide. NOD.NON.*Thy1*^{a} mice were injected i.v. with (*A*) 2×10^6 CFSE-labeled AI4 T cells purified from NOD.*AI4*$\alpha\beta$Tg mice or (*B*) 2×10^6 CFSE-labeled CD8$^+$ T cells purified from NOD.*LCMV TCR Tg* mice. Twenty-four hours later, recipient mice were treated i.p. with (*A*) 10 μg of anti-DEC-205/MimA2 or control Ig/MimA2 or PBS, or (*B*) 10 μg of anti-DEC-205/GP$_{33-41}$ or PBS. Three days after hybrid antibody injection, lymphoid organs were harvested, and the proliferation of Thy1.2$^+$ CD8$^+$ cells was evaluated by flow cytometry for CFSE dilution. Data shown are representative of at least two experiments.

in their pancreatic lymph nodes because of endogenous presentation of AI4's natural β cell antigen at this site (26).

Because AI4 T cells recognize a β cell antigen and cause T1D in NOD.*AI4*$\alpha\beta$ Tg mice (24), it is possible that the transferred T cells had previously been exposed to their antigen and were not naive. Furthermore, at least some of the transferred cells were clearly exposed to their natural antigen in the pancreatic lymph nodes of the recipient mice (Fig. 2*A*). To confirm that peptide-linked anti-DEC-205 is alone sufficient to stimulate the proliferation of NOD T cells, even if naive, we used T cells from NOD.*LCMV TCR Tg* mice (27), which are specific for a peptide derived from lymphocytic choriomeningitis virus (LCMV) glycoprotein (GP$_{33-41}$) presented by H-2D^b. These mice serve as a source of purely naive splenocytes, because the LCMV protein is not expressed in these animals. We transferred CFSE-labeled Thy1.2$^+$ CD8$^+$ LCMV-specific T cells to NOD.NON-*Thy1*^{a} recipients and treated the recipients with anti-DEC-205/GP$_{33-41}$. As shown in Fig. 2*B*, after 3 d, extensive T cell proliferation was observed in the peripheral lymph nodes, pancreatic lymph nodes, and spleens of the treated mice (with 51 ± 28%, 59 ± 13%, and 54 ± 15% of cells showing CFSE dilution, respectively; $n = 2$) but not in PBS-injected controls (1.1 ± 0.18%, 1.0 ± 0.099%, and

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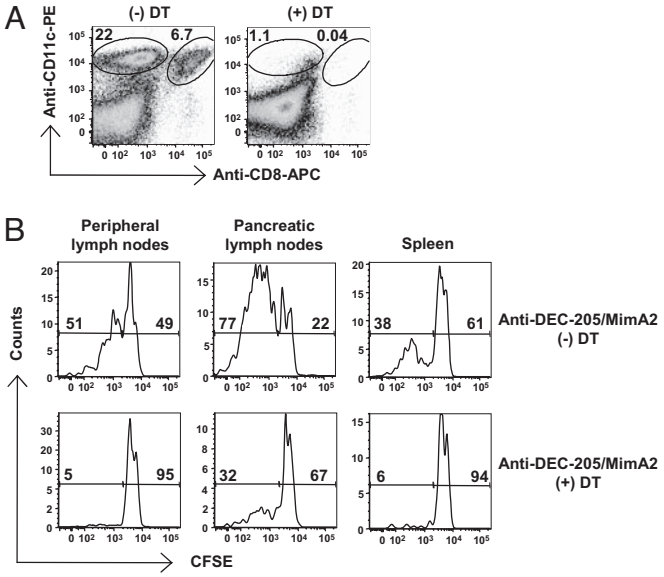

Fig. 3. *In vivo* DC depletion blocks CD8$^+$ T cell proliferation in response to anti-DEC-205/MimA2. NOD.CD11c-DTR.*Thy1*^{a} mice were injected i.v. with 2×10^6 CFSE-labeled AI4 T cells and 16 h later with 4 ng/g DT i.p. or PBS. Eight hours after the DT or PBS injection, recipient mice were given 10 μg of anti-DEC-205/MimA2 i.p. (*A*) Three days after antibody treatment, spleens were dissociated with collagenase D and the CD11c$^+$ CD8$^+$ and CD11c$^+$ CD8$^-$ DC subsets were identified after enrichment into a low-density cell fraction and analysis by flow cytometry, with samples gated on B220$^-$ CD3$^-$ cells. (*B*) Lymphoid organs were harvested to evaluate the proliferation of Thy1.2$^+$ CD8$^+$ AI4 T cells by flow cytometry for CFSE dilution. Data shown are representative of two similar experiments.

1.2 $\pm$ 0.54%). These results indicate that peptide-linked anti-DEC-205 is alone sufficient to induce the proliferation of naive NOD T cells, even when the dose of injected peptide is relatively small, <20 ng per mouse.

DCs Are Required for the *in Vivo* Activity of Antigen-Linked Anti-DEC-205 Antibodies. Although DEC-205 is expressed at high levels by a subset of DCs, it is also expressed by a number of other cell types *in situ* such as thymic and intestinal epithelia, follicular B cells, bone marrow stromal cells, and pulmonary airway epithelia (7). To establish the importance of DCs here, we developed NOD.CD11c-DTR.*Thy1*^{a} mice. These mice carry a transgene encoding a simian diphtheria toxin (DT) receptor (DTR)-GFP fusion protein under the control of the murine *CD11c* promoter, which is active in nearly all murine DC subsets. As reported for FVB/N mice carrying this transgene (28), use of 4 ng/g of DT selectively depleted CD11c$^+$ cells in the NOD.CD11c-DTR mice (Fig. 3*A*), and the toxin-induced DC depletion persisted for 2 d, after which DC numbers were gradually restored (data not shown). It has been reported that marginal zone macrophages are also depleted upon DT treatment in mice expressing the CD11c-DTR transgene (29); however, because these cells do not express DEC-205 (7), they need not be considered here. CFSE-labeled Thy1.2$^+$ AI4 cells (2×10^6) were injected i.v. into NOD.CD11c-DTR.*Thy1*^{a} mice, and 16 h later, we injected 4 ng/g DT i.p. followed 8 h later by 10 μg of anti-DEC-205/MimA2 i.p. After 3 d, T cell proliferation was found to be dramatically reduced in the lymphoid tissues of DT-treated mice (Fig. 3*B*), indicating that DCs are required for DEC-205-mediated cross-presentation of antigen via class I MHC. Some proliferation of transferred AI4 T cells was observed in the pancreatic lymph nodes of DT-treated mice but not in the peripheral lymph nodes or spleen. This is likely due to partial recovery of DCs 3 days

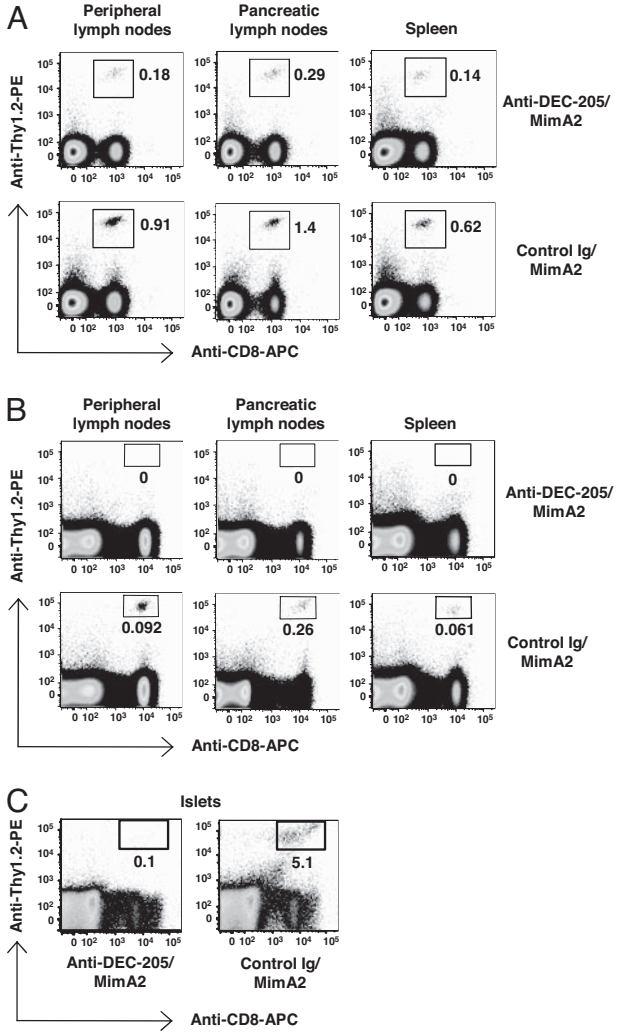

Fig. 4. Anti-DEC-205/MimA2 targeting of DCs *in vivo* leads to deletion of AI4 T cells. (*A*) NOD.NON.*Thy1*^{a} mice were injected i.v. with 2×10^6 AI4 T cells from NOD.*AI4*$\alpha\beta$Tg mice and, after 24 h, i.p. with 10 μg of anti-DEC-205/MimA2 or control Ig/MimA2. Twelve days later, lymphoid organs of recipient mice were harvested, and Thy1.2$^+$ CD8$^+$ cells were enumerated by flow cytometry. (*B*) Same as in *A*, but the transferred T cells were from NOD.*Rag1*^{null}. *AI4*$\alpha\beta$Tg mice. (*C*) The pancreata of the same mice used in *B* were perfused and islets were picked and cultured for 2 d. Islet infiltrates were harvested and evaluated for Thy1.2$^+$ CD8$^+$ cells by flow cytometry. In *A-C*, numbers denote the percentage of CD8$^+$ cells present in the indicated gates. Data shown are representative of at least two experiments.

after DT treatment, as has been reported by others (28). The proliferation is selective for the pancreatic lymph node, because presentation of AI4's endogenous β cell antigen occurs only at this site (Fig. 2*A Lower*).

Transferred β Cell-Autoreactive CD8$^+$ T Cells Are Eventually Deleted in Response to Antigen Targeting via DEC-205. To test whether treatment of NOD mice with anti-DEC-205/MimA2 would result in the eventual deletion of transferred AI4 T cells, we again transferred these cells to NOD.NON-*Thy1*^{a} mice and injected either anti-DEC-205/MimA2 or control Ig/MimA2. This time, however, we looked for the AI4 T cells after 12 rather than 3 d (Fig. 4*A*). In the peripheral lymph nodes, pancreatic lymph nodes, and spleens of mice treated with anti-DEC-205/MimA2, the transferred AI4 T cells constituted a smaller fraction of all CD8$^+$ cells compared with control Ig/MimA2-

treated animals. This analysis indicated that AI4 T cells were largely, although not completely, deleted as a result of anti-DEC-205/MimA2 treatment.

It has been reported that approximately one-quarter of the $CD8^+$ T cells in NOD.*AI4*$\alpha\beta$Tg mice express the transgenic TCR α chain at a greatly reduced level because of coexpression of endogenous α chains (20). We reasoned that such T cells would respond poorly, if at all, to MimA2 and would fail to be deleted in response to treatment with anti-DEC-205/MimA2. In support of this idea, when we transferred purified $CD8^+$ T cells from NOD.*Rag1*^{null}.*AI4*$\alpha\beta$Tg mice, we observed essentially complete deletion of transferred cells from lymph nodes and spleen after 12 d in response to anti-DEC-205/MimA2 treatment (Fig. 4*B*). In the mice treated with anti-DEC-205/MimA2, the transferred cells constituted $0.0014 \pm 0.0024\%$ of $CD8^+$ cells in peripheral lymph nodes, $0.0012 \pm 0.0018\%$ in pancreatic lymph nodes, and $0.0014 \pm 0.0024\%$ in spleen ($n = 3$). The corresponding values for control Ig/MimA2-treated mice were $0.076 \pm 0.021\%$, $0.17 \pm 0.078\%$, and $0.063 \pm 0.0053\%$, respectively.

As it was possible that the transferred AI4 T cells had migrated to the islets rather than undergoing true deletion, we isolated the pancreatic islets on day 12 and cultured the islet infiltrate for 2 d. The cells were then harvested, stained for Thy1.2 and CD8, and analyzed by flow cytometry (Fig. 4*C*). In control Ig/MimA2-treated animals, we observed enrichment of the transferred cells in the islet infiltrate, where they constituted $8.6 \pm 5.0\%$ of $CD8^+$ T cells ($n = 2$), whereas in mice treated with anti-DEC-205/MimA2, few transferred cells were observed in the infiltrate ($0.096 \pm 0.0057\%$). These findings confirmed that AI4 T cells were largely deleted as a result of anti-DEC-205/MimA2 treatment.

Antigen Targeting to DCs via DEC-205 Induces Peripheral $CD8^+$ T Cell Tolerance. A critical criterion for the induction of tolerance is the inability to respond to rechallenge with antigen delivered together with a strong adjuvant. To determine whether anti-DEC-205/MimA2-treated NOD mice became tolerant to MimA2, we transferred 2×10^6 Thy1.2$^+$ $CD8^+$ AI4 T cells or Thy1.2$^+$ $CD8^+$ *Rag1*^{null} AI4 T cells to NOD.NON-*Thy1*^{a} mice. Twelve days after administration of 10 μg of anti-DEC-205/MimA2, mice were immunized with 50 μg of MimA2 peptide suspended in CFA. Three days after CFA/peptide immunization, the peripheral lymph node cells were harvested, and the immune response was evaluated by intracellular cytokine staining for IFN-γ production in response to *in vitro* stimulation with MimA2. IFN-γ-producing cells were not detectable from mice treated 12 d earlier with anti-DEC-205/MimA2, regardless of whether the transferred AI4 cells were from *Rag1*-positive (Fig. 5*A*) or -negative donors (Fig. 5*B*). In contrast, mice treated with PBS (data not shown), control Ig/MimA2 (Fig. 5 *A* and *B*), or a combination of anti-DEC-205/MimA2 and immunogenic DC maturation stimuli (anti-CD40 and poly I:C) (Fig. 5*B*) produced strong IFN-γ responses. Thus, targeting of MimA2 to DCs via DEC-205 results in T cell tolerance, provided the antigen is not administered in combination with DC maturation stimuli.

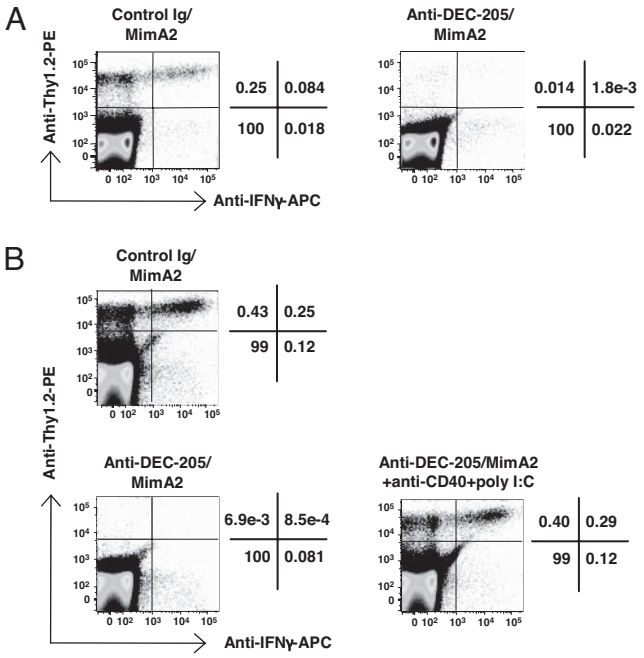

Fig. 5. *In vivo* targeting of DCs with anti-DEC-205/MimA2 induces tolerance to MimA2. (*A*) NOD.NON.*Thy1*^{a} mice were injected i.v. with 2×10^6 AI4 T cells from NOD.*AI4*$\alpha\beta$Tg mice and i.p. with 10 μg of anti-DEC-205/MimA2 or control Ig/MimA2. Twelve days after hybrid antibody administration, mice were boosted with 50 μg of MimA2 peptide in CFA. Three days later, peripheral lymph nodes were harvested and stimulated with free MimA2 peptide and AI4 T cells evaluated for production of intracellular IFN-γ. (*B*) Same as in *A*, but the transferred T cells were from NOD.*Rag1*^{null}.*AI4*$\alpha\beta$Tg mice and the recipient mice were given anti-DEC-205/MimA2 i.p. with or without anti-CD40 (25 μg) and poly I:C (50 μg).

Discussion

Bonifaz *et al.* (13) reported that treatment of C57BL/6 mice with anti-DEC-205 chemically coupled to the ovalbumin protein causes proliferation of transferred ovalbumin-specific $CD8^+$ T cells, followed by the establishment of deletional tolerance. However, for several reasons, it was unclear whether similar findings would be obtained upon treatment of NOD mice with anti-DEC-205/MimA2. First, deficiencies in central and peripheral tolerance mechanisms, including defects in $CD8^+$ T cell peripheral tolerance, have been described in NOD mice (20–23). Lack of response to a transplantation tolerance induction protocol has similarly been reported for this strain (30), and DC defects have also been noted (9–12). Furthermore, the T1D-susceptible NOD mouse recipients used in our work were mounting immune responses to β cell antigens, including AI4's target, during the experimental period, as evidenced by infiltrating autoreactive T cells in their islets (Fig. 4*C*). This is a very different situation from that present in the nonautoimmune-prone mice used by Bonifaz *et al.* (13). Also, in this previous work, the antigen under study was a foreign one, virtually ensuring that the transferred ovalbumin-specific T cells would be naive. This is unlikely to be the case in our experiments, where the AI4 T cells are harvested from T1D-susceptible NOD.*AI4*$\alpha\beta$Tg mice. As a result, our finding that DCs can mediate T cell tolerance in NOD mice with known DC and tolerance defects could not have been predicted from the existing literature. Furthermore, the finding that DCs can mediate T cell tolerance even in the context of a spontaneous, ongoing autoimmune condition could have significant biological and therapeutic implications.

When Hogquist and coworkers (31) engineered C57BL/6 mice in which a class I MHC-binding peptide was presented by both activating and tolerogenic DCs in a single animal, transferred peptide-specific T cells failed to accumulate, indicating that tolerance dominated. We speculate that, because anti-DEC-205/MimA2 gained access to peripheral lymph nodes and spleen in addition to the pancreatic lymph nodes in our experiments (Fig. 2), tolerance was similarly able to predominate over ongoing autoimmunity. The mechanism(s) responsible for this tolerance induction will be the subject of future work. In particular, we hypothesize that the PD-1 pathway (32) may be participating in this process. This is because van den

Broek and coworkers (19), using DIETER (DC-specific inducible expression of T cell epitopes by recombination) mice, showed that peripheral $CD8^+$ T cell tolerance induced by steady-state DCs is T cell-intrinsic and deletional and requires PD-1 on T cells (19).

With our currently available anti-DEC-205 reagents, it is not feasible to monitor the impact of peptide-linked anti-DEC-205 treatment on endogenous T cells (as opposed to transferred ones) in NOD mice, because the low prevalence of the MimA2 specificity (33, 34) would make it very difficult to draw meaningful conclusions. Endogenous T cells will be examined once we have developed reagents to target cells specific for residues 206–214 of islet-specific glucose-6-phosphatase catalytic subunit-related protein, which is a far more prevalent specificity (33, 34). Clearly, prevention and intervention studies will also need to be conducted in mice before exploration of antigen-linked anti-DEC-205 as a potential therapeutic strategy for human T1D. Our next step will involve optimization of multiple parameters (including antibody dose, number of treatments, and the time relative to T cell transfer at which antibody treatment is begun) and determination of the ability of anti-DEC-205/MimA2 therapy to prevent T1D in an adoptive transfer model of AI4-induced disease (26).

In summary, NOD mice have a variety of defects that interfere with the establishment or maintenance of T cell tolerance and lead to the spontaneous development of T1D (20–23). Here, we demonstrate that targeting of a peptide antigen to $DEC\text{-}205^+$ DCs *in vivo* allows these defects to be overcome, even in the context of ongoing β cell autoimmunity, and permits the establishment of deletional tolerance.

Materials and Methods

Mice. NOD.*AI4*$\alpha\beta$ Tg (24) and NOD.*Rag1*^{null}.*AI4*$\alpha\beta$Tg mice (35) express the TCR from the NOD-derived H-$2D^b$-restricted diabetogenic $CD8^+$ T cell clone AI4 (24). NOD.*LCMV TCR Tg* mice (27) express an H-$2D^b$-restricted TCR specific for LCMV $GP_{33–41}$. Fully T1D-susceptible NOD.NON-*Thy1*^{a} mice have been described (36). To generate a NOD stock carrying a DTR transgene driven by the *CD11c* promoter, B6.CD11c-DTR mice (28) were backcrossed to NOD for at least eight generations. An *Idd* sweep was performed, and the congenic region linked to the transgene was found to partially overlap *Idd5*. Linkage markers delineating all other known *Idd* loci have been fixed to homozygosity for NOD alleles in the strain now designated NOD.CD11c-DTR. Intercrossing of NOD.CD11c-DTR and NOD.NON-*Thy1*^{a} mice was used to obtain the NOD.CD11c-DTR.*Thy1*^{a} strain used for the current studies. All mice were bred under specific pathogen-free conditions at the Albert Einstein College of Medicine following protocols approved by the Institutional Animal Care and Use Committee.

Hybrid Antibody Production. Previously, the V regions of NLDC-145 (rat anti-mouse DEC-205) and GL117 (an isotype match for NLDC-145) were cloned in frame with the mouse Igκ or mouse IgG_1 constant region having mutations that interfere with FcR binding (15). To create anti-DEC-205/MimA2 and control Ig/MimA2, we fused these previously described heavy chains to MimA2 (YAIENYLEL) (25) flanked by the sequences naturally flanking the H-$2K^b$-binding ovalbumin peptide SIINFEKL. Using synthetic oligonucleotides (5′-CTAGCGACATGGCCAAGAAGGAGACAGTCTGGAGGCTCGAGGAGTTCG-GTAGG-3′, 5′-ACTCCTCGAGCCTCCAGACTGTCTCCTTCTTGGCCATGTCG-3′, 5′-GAGCAGCTTGAGTACGCCATCGAGAACTACCTGGAGCTGACTGAATG-GACTCCAGGCAAGTAAGC-3′, 5′-GGCCGCTTACTTGCCTGGAGTCCATTCAGTC-AGCTCCAGGTAGTTCTCGATGGCGTACTCAAGCTGCTCCCTACCGA-3′), MimA2 and these flanking regions were cloned in frame with the C terminus of the heavy chain of anti-DEC-205. Anti-DEC-205/$GP_{33–41}$ was similarly prepared by using oligonucleotides encoding LCMV $GP_{33–41}$ (KAVYNFATC). Hybrid antibodies were produced by transient transfection of 293T cells by using calcium phosphate as described in ref. 15. Cells were grown in serum-free DMEM supplemented with Nutridoma SP (Roche). Seven to 10 d after transfection, antibodies were purified on Protein G affinity columns (Amersham Pharmacia). Antibody concentrations were determined by the Bradford method (BioRad) by using bovine γ-globulin as a standard.

Antibodies. Monoclonal antibodies to murine Thy1.1 (OX-7), Thy1.2 (53–2.1), CD8α (53–6.7), CD40 (HM40–3), CD3 (145–2C11), B220 (RA3–6B2), and IFN-γ (XMG1.2) and polyclonal goat anti-mouse IgG + IgM were purchased from BD Biosciences. Magnetic microbeads and antibodies for purification of $CD11c^+$ and $CD8^+$ cells were purchased from Miltenyi Biotec.

Isolation of DCs and *in Vitro* T Cell Proliferation Assay. Spleens were removed from adult female NOD mice. Single-cell suspensions were prepared with 400 units/ml collagenase D for 25 min (Roche). After low-density cell enrichment using 30% BSA (Sigma–Aldrich), the cells were incubated with anti-mouse CD11c MACS microbeads (Miltenyi Biotec) for 30 min on ice. $CD11c^+$ (DC-enriched) and $CD11c^-$ cells were separated by application of a magnetic field and dispensed into 96-well plates on day 0. Cells were cultured with anti-DEC-205/MimA2 or control Ig/MimA2 overnight (16–20 h) at 37°C. On day 1, $CD8^+$ AI4 T cells were purified from single-cell suspensions of NOD.*AI4*$\alpha\beta$Tg mouse spleens by negative selection using MACS microbeads (Miltenyi Biotec). T cells (5×10^4) were added to graded doses of $CD11c^+$ or $CD11c^-$ cells in round-bottom 96-well plates. [^{3}H]thymidine (1 μCi; GE Healthcare) was added 72 h later, and its incorporation into DNA was measured after 8–16 h by using an automated cell harvester.

***In Vivo* T Cell Proliferation and Deletion Assays.** $CD8^+$ AI4 T cells were purified from spleens of NOD.*AI4*$\alpha\beta$Tg or NOD.*Rag1*^{null}.*AI4*$\alpha\beta$Tg mice (as indicated) and incubated at 10^7 cells per ml with 5 μM CFSE (Invitrogen) for 10 min at 37°C. An equal volume of FCS (Invitrogen) was added and the cells washed two times with PBS/0.1% BSA and twice with PBS alone. Labeled AI4 T cells (2×10^6) were injected i.v. into female NOD.NON.*Thy1*^{a} recipients (6–8 weeks of age). The following day, the recipient mice were injected i.p. with 10 μg of anti-DEC-205/MimA2, 10 μg of control Ig/MimA2, or PBS. Peripheral (popliteal, inguinal, axillary, and brachial) and pancreatic lymph nodes and spleens were harvested 3 or 12 d later. Transferred cells were identified by positive staining for both Thy1.2 and CD8. On day 3, proliferation of CFSE-labeled T cells was assessed by progressive halving of the amount of CFSE fluorescence per cell. Samples were evaluated by multicolor flow cytometry by using a FACSCalibur or LSRII (BD Biosciences) with subsequent analysis of data in FlowJo (Tree Star).

Also on day 12, pancreata were harvested and digested with collagenase P, and islets were hand-picked into 24-well plates (50 islets per well) and cultured in RPMI medium 1640 supplemented with 50 units/ml hIL-2 to permit T cells to exit from the islets. Two days later, the cells were harvested, passed through a 40-μM mesh, washed twice, stained for Thy1.2 and CD8, and monitored by multicolor flow cytometry by using an LSRII.

In vivo T cell proliferation experiments were also performed by using anti-DEC-205/$GP_{33–41}$ and $CD8^+$ T cells purified from the spleens of NOD.*LCMV TCR Tg* mice as described above for AI4.

***In Vivo* DC Depletion and Anti-DEC-205 Targeting in DC-Depleted Mice.** $Thy1.2^+$ AI4 cells were purified from the spleens of NOD.*AI4*$\alpha\beta$ Tg mice as above and labeled with CFSE, and 2×10^6 cells were injected i.v. into NOD.CD11c-DTR.*Thy1*^{a} mice. Sixteen hours later, recipients received either 4 ng/g DT (Sigma–Aldrich) i.p. or PBS. Eight hours after the DT injection, all recipients received an i.p. injection of 10 μg of anti-DEC-205/MimA2. After 3 d, lymph nodes (peripheral and pancreatic) and spleens were evaluated for AI4 proliferation by CFSE dilution as described above.

Determination of the Establishment of Tolerance. Purified AI4 $CD8^+$ T cells (2×10^6) were transferred to female NOD.NON-*Thy1*^{a} mice (6–8 weeks of age). The next day, anti-DEC-205/MimA2 was injected with or without an i.p. injection of anti-CD40 (25 μg of per mouse) and poly I:C (50 μg per mouse; Sigma–Aldrich). Twelve days after antigen injection, mice were boosted with MimA2 peptide (50 μg s.c.) suspended in CFA (Sigma–Aldrich). Three days later, 5×10^6 peripheral lymph node cells were pulsed with the MimA2 peptide for 5 h, then brefeldin A (BD Biosciences) was added and incubated for 6 h. Cells were then harvested, washed twice with PBS/2% FBS, and stained for extracellular Thy1.2. Cells were next fixed and stained for intracellular IFN-γ after permeabilizing the cell with buffer containing saponin (Sigma–Aldrich). Flow cytometry was performed as described above.

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