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CD8$^+$ CD122$^+$ PD-1$^-$ effector cells promote the development of diabetes in NOD mice

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ABSTRACT

It is well established that CD4 and CD8 T cells are required for the initiation of autoimmune diabetes in NOD mice. However, different subsets of CD4 or CD8 cells may play different roles in the initiation of insulitis. In this study, we evaluated the role of the previously described CD8$^+$ CD122$^+$ in this process. We found that prediabetic NOD mice have an almost 50% reduction of CD8$^+$ CD122$^+$ T cells in their secondary lymphoid organs compared with BL/6 or Balb/c mouse strains. This reduction is explained by the lack of the regulatory CD8$^+$ CD122$^+$ PD-1$^+$ cell population in the NOD mice, as we found that all CD8$^+$ CD122$^+$ T cells from prediabetic NOD mice lack PD-1 expression and regulatory function. Depletion of CD8$^+$ CD122$^+$ PD-1$^-$ cells through injection of anti-CD122 mAb in prediabetic female NOD mice reduced the infiltration of mononuclear cells into the Langerhans islets and delayed the onset and decreased the incidence of overt diabetes. In addition, we found that transfer of highly purified and activated CD8$^+$ CD122$^+$ PD-1$^-$ cells, together with diabetogenic splenocytes from NOD donors to NOD SCID recipients, accelerates the diabetes development in these mice. Together, these results demonstrate that CD8$^+$ CD122$^+$ PD-1$^-$ T cells from NOD mice are effector cells that are involved in the pathogenesis of autoimmune diabetes. *J. Leukoc. Biol.* 97: 111–120; 2015.

Introduction

The NOD mouse develops spontaneous autoimmune diabetes and represents a good model for human T1D [1, 2]. Aside from developing T1D, NOD mice exhibit a number of other autoimmune polyendocrine manifestations, notably, sialitis and thyroiditis.

In recent years, several cellular mechanisms have been identified as playing a role in T1D development in the NOD mouse, including cytotoxic CD8$^+$ lymphocytes [3–5], B lymphocytes [4], Th17 cells [6], as well as dysregulation of the Treg compartment [7, 8]. In female mice, overt disease spontaneously appears mostly by 12–16 weeks of age when mononuclear cells invade the islets and initiate the destruction of insulin-producing β cells (invasive insulitis). This phase is preceded by a long phase of asymptomatic "prediabetes," characterized by progressive insulitis, starting as early as 3 weeks of age. In this initial phase, mononuclear cells, including CD4$^+$ and CD8$^+$ lymphocytes, infiltrate only the periphery of the Langerhans islets (peripheral insulitis). Progression to overt diabetes is accompanied by invasive, intraislet insulitis, and hyperglycemia occurs when 70–80% of the β-cell mass is destroyed.

It has been shown that the spontaneous development of diabetes in NOD mice requires the functional activity of both CD4$^+$ and CD8$^+$ T cells. Successful transfer of T1D by T cells from a diabetic NOD donor mouse to lymphopenic recipients (such as NOD SCID mice) has also been shown to require both subsets of T cells [9–12]. In agreement with these data, it has been reported that the NOD-derived, islet-reactive CD4$^+$ T cell clone BDC-2.5 is only able to transfer diabetes to adult NOD or NOD SCID recipients when transferred together with CD8$^+$ splenic T cells from a diabetic donor [13, 14]. In contrast, CD8-depleted splenic cells from a diabetic donor were not able to transfer diabetes on their own. This suggests that CD8$^+$ T cells may play a functional role in the induction of β cell destruction [14, 15].

However, the CD8 compartment comprises of a variety of subpopulations, and it is not clear which CD8 subpopulation is required for the progression of insulitis.

It is well established that CD8$^+$ CD122$^+$ T cells are antigen-specific memory/effector T cells [16–18]. Moreover, it has been shown that CD8$^+$ CD122$^+$ T cells produce larger amounts of IFN-γ than other T or NKT cells upon CD3 stimulation in vitro and acquire a significant antitumor cytotoxicity [19, 20]. Thus, it could be suggested that this CD8 subpopulation also contributes to the development of autoimmune diseases. On the other hand, recent studies have identified naturally occurring CD8$^+$CD122$^+$

Abbreviations: BL/6 = C57BL/6, CD62L = cluster of differentiation 62 ligand, Foxp3 = forkhead box p3, IGRP = islet-specific glucose-6-phosphatase catalytic subunit-related protein, PD-1 = programmed death-1, T1D = Type 1 diabetes, Treg = regulatory T cell

The online version of this paper, found at www.jleukbio.org, includes supplemental information.

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T cells as Tregs that maintain T cell homeostasis and suppress T cell responses [21–23].

In this study, we sought to determine the role of naturally occurring $CD8^+$ $CD122^+$ T cells in the development of autoimmune diabetes in the NOD mice. We found that $CD8^+$ $CD122^+$ T cells are reduced in the secondary lymphatic organs in prediabetic NOD female mice and lack regulatory function. Depletion of $CD122^+$ cells led to a decreased incidence of overt diabetes, and accordingly, the cotransfer of highly purified $CD8^+$ $CD122^+$ cells together with splenocytes accelerate diabetes development.

MATERIALS AND METHODS

Mice

Female NOD mice were purchased from The Jackson Laboratory (Bar Harbor, ME, USA), BL/6 and Balb/C mice were purchased from Harlan-Winkelmann (Borchen, Germany). and NOD SCID mice (The Jackson Laboratory) were bred and maintained in our animal facility. All experiments were performed under the approval and in accordance with the guidelines of the Bavarian animal welfare authority. All mice were housed in a specific pathogen-free condition. Blood glucose levels were monitored twice/week by use of OneTouch Ultra (LifeScan, Neckargemünd, Germany) glucometers. Diabetes in NOD female mice was defined as 2 consecutive blood glucose values >250 mg/dl.

Flow cytometry

Single-cell suspensions were prepared from the thymus, spleen, peripheral lymph nodes, and peripancreatic lymph nodes, as described previously [24]. The pancreas was dissected from the peripancreatic lymph nodes and cut into pieces. The pancreas was digested into single-cell suspensions by incubation with RPMI 1640 containing 4 mg/10 ml collagenase P (Roche, Indianapolis, IN, USA) for 30 min at 37°C and then washed with 14 ml RPMI 1640, supplemented with 10% FCS. Subsequently, 1×10^6 cells were stained with different mAb for 15 min at 4°C, washed, and then analyzed on a FACSCanto II by use of FlowJo software (BD Biosciences, Heidelberg, Germany). Antibodies were purchased from eBioscience (San Diego, CA, USA). Single-cell suspensions from the pancreas were prepared as followed.

Intracellular staining

Intracellular Foxp3, IFN-γ, granzyme B, and perforin staining was performed by use of a fixation and permeabilization kit (eBioscience), according to the manufacturer's instructions. To measure IFN-γ, granzyme B production after in vitro cell stimulation, cytokine secretion was blocked by use of the protein transport inhibitor brefeldin A (eBioscience), according to the manufacturer's instructions.

Depletion of $CD122^+$ cells

Depletion of $CD122^+$ cells in NOD female mice was achieved by i.v. injection of 50 μg anti-CD122 mAb (TM-β1) or IgG2b isotype control mAb (eBMG2b; both antibodies were purchased from eBioscience) on d 0, 14, 28, 42, and 56. To deplete the $CD122^+$ cells before cell transfer, NOD female mice received i.v. injection of 50 μg anti-CD122 mAb (TM-β1) on d −3 (cell transfer of splenocytes into NOD SCID mice: d 0). Successful depletion of $CD122^+$ cells was determined by FACS by use of anti-CD122 antibodies (clone 5H4) that recognize a different epitope than the TM-β1 clone to exclude epitope masking.

Adoptive transfer

Diabetes was transferred to 5- to 9-week-old NOD SCID mice by i.v. injection of 5×10^6 spleen cells isolated from female diabetic NOD mice. One group of NOD SCID mice received 5×10^6 spleen cells from female diabetic NOD mice that were treated 3 d earlier with i.v. injection of 50 μg anti-CD122 mAb (TM-β1) to deplete $CD122^+$ before cell transfer.

Freshly isolated $CD8^+CD122^+$ cells (1×10^6) obtained from female nondiabetic NOD mice were cultured in anti-CD3 mAb-coated 96-well plates (clone 145-2C11; eBioscience) with a medium containing recombinant mouse IL-2 (10 ng/ml; PeproTech, Rocky Hill, NJ, USA). Forty-eight hours later, these activated $CD8^+CD122^+$ cells were transferred together with 5×10^6 spleen cells from female diabetic NOD mice into NOD SCID mice, and diabetes development was evaluated.

T cell isolation and cell sorting

Single-cell suspensions of splenic tissue were prepared, and $CD4^+$ or $CD8^+$ T cells were negatively selected by use of the mouse $CD4^+$ or $CD8a^+$ T Cell Isolation Kit II, MACS (Miltenyi Biotec, Bergisch-Gladbach, Germany). To isolate $CD122^+$ or $CD122^-$ T cells from the purified $CD8^+$ cell population, $CD8^+$ cells were incubated with anti-CD122-PE–labeled antibody (clone 5H4; eBioscience). $CD8^+$ $CD122^+$ and $CD8^+CD122^-$ T cells were sorted out with a FACSAria cell sorter (BD Biosciences). The purity of $CD8^+$ $CD122^+$ or $CD8^+$ $CD122^-$ cells was >96%

Proliferation assay

Splenic-purified $CD8^+$ $CD122^+$ or $CD8^+$ $CD122^-$ T cells (2×10^5 cells/well) were labeled with 0.1 μM CFSE (Invitrogen, Karlsruhe, Germany) for 10 min at 37°C. Equal loading was confirmed by flow cytometry. Subsequently, cells were cultured in RPMI-1640 medium (supplemented with 10% FCS, antibiotics, and 2-ME; Invitrogen) and left unstimulated or activated with plate-bound anti-CD3 (clone 145-2C11; eBioscience; 1 μg/ml) and with soluble anti-CD28 mAb (clone 37.51; eBioscience; 5 μg/ml) [25]. Cells were cultured for 72 h at 37°C with 5% CO_2. Proliferation was assessed by CFSE dilution analysis.

Suppression assay

As $CD4^+$ T cells are known to be regulated by $CD8^+$ $CD122^+$ Tregs [21], we used $CD4^+$ $CD25^-$ T cells from NOD female mice as responder cells. For suppression assays, sorted $CD8^+$ $CD122^-$ or $CD8^+$ $CD122^+$ (2×10^6) from NOD female mice was stimulated for 48 h with plate-bound anti-CD3 (1 μg/ml) and soluble anti-CD28 (5 μg/ml). After these 48 h, activated $CD8^+$ $CD122^+$ suppressor cells or $CD8^+$ $CD122^-$ cells were added to CFSE-labeled $CD4^+$ $CD25^-$ responder cells in different ratios (suppressor:responder ratio, 1:5 and 1:1) and cultured with anti-CD3 (1 μg/ml) and anti-CD28 (5 μg/ml) in a 96-well, flat-bottom plate. Seventy-two hours later, the proliferation of the CFSE-labeled $CD4^+$ $CD25^-$ responder cells was assessed by CFSE dilution analysis.

Histology

Pancreas were fixed in 4% paraformaldehyde solution (Sigma-Aldrich, Munich, Germany) and processed for paraffin embedding. Sections (5 μm thick) were stained with H&E, and the degree of insulitis was evaluated microscopically, as described previously [26]. At least 5 mice and 170–290 islets/group were analyzed. Insulitis was scored as follows: 1 = no infiltration, 2 = peri-insulitis, 3 = mild infiltration, and 4 = severe infiltration (>70% mononuclear cells/islet).

Statistical analysis

Statistical significances were calculated by use of the two-tailed unpaired Student's *t*-test with 95% confidence intervals. Comparisons of groups (see Figs. 4B, 6, and 7) were analyzed by log-rank Mantel-Cox test.

RESULTS

Prediabetic NOD female mice have reduced $CD8^+$ $CD122^+$ T cells in secondary lymphoid organs

Previous studies have shown no significant differences in the size of peripheral T cell compartments between NOD and other

mouse strains, such as BALB/c, BL/6, and CBA mice [27]. However, a detailed analysis of the CD8$^+$ CD122$^+$ T cell pool has not yet been determined. Therefore, we analyzed the CD8$^+$ CD122$^+$ T cells from spleen, peripheral lymph nodes, and peripancreatic lymph nodes of NOD mice who were 1 week and 10–12 weeks old, respectively. Flow cytometry was used to compare the cells with the CD8$^+$ CD122$^+$ T cells of BALB/c and BL/6 mice. As shown in **Fig. 1A** and **C** and Supplemental Fig. 1, 1-week-old NOD female mice possess a very small population of CD8$^+$ CD122$^+$ T cells in their secondary lymphatic organs. The size of this population is comparable with 1-week-old female BALB/c and B6 mice. Furthermore, the percentage distribution of CD122$^+$ cells within the CD8$^+$ cell pool was similar. A more detailed analysis discovered that CD8$^+$ CD122$^+$ T cells are almost all CD62L and CD44 positive (Fig. 1B). Therefore, they resemble those CD8$^+$ CD122$^+$ that are found in BALB/c and BL/6 mice.

Next, we determined the CD8$^+$ CD122$^+$ T cell pool in prediabetic female NOD mice compared with age-matched, diabetes-resistant mouse strains. As shown in Fig. 1B and C, the population of CD8$^+$ CD122$^+$ T cells was reduced significantly by 50–60% in the spleen, as well as in the peripheral and peripancreatic lymph nodes, compared with BALB/c and BL/6 mice. However, similar to the results found in 1-week-old mice, all CD8$^+$ CD122$^+$ cells of prediabetic NOD mice also express CD44 and CD62L (Fig. 1B). The expression intensity of these markers among CD8$^+$ CD122$^+$ T cells was equal between NOD female mice and BALB/c and BL/6 female mice. With the consideration of this decrease in the CD8$^+$ CD122$^+$ cell population in 10- to 12-week-old mice, we investigated this cell population again in 3- and 5-week-old mice. Figure 1C shows that the CD8$^+$ CD122$^+$ cell population increases over age in BL/6 and BALB/c mice. In contrast, we found that the splenic, peripancreatic, and lymphatic CD8$^+$ CD122$^+$ cell population in NOD mice increases until the age of 3 weeks, after which, it remains stable. In contrast, this cell population does not increase in older NOD mice, such as BL/6 and BALB/c strains. Ex vivo analyzation of Annexin V staining revealed no differences, indicating that loss of this cell population was not a result of enhanced apoptosis of these cells in NOD mice (Supplemental Fig. 2).

These data indicate that the number and percentage of CD8$^+$ CD122$^+$ CD44$^+$ CD62L$^+$ T cells in the secondary lymphoid organs are normal in NOD female mice during the postnatal period, implying that the NOD mice do not have a developmental defect of CD8$^+$ CD122$^+$ T cells. However, this cell population does not increase over age in NOD female mice, as observed in BL/6 mice and BALB/c mice.

CD8$^+$ CD122$^+$ PD-1$^-$ T cells from NOD mice possess effector function

In light of the above results, we subsequently investigated the reason for CD8$^+$ CD122$^+$ cell population reduction in 10-week-old NOD mice. A recent publication suggested that CD8$^+$ CD122$^+$ T cells could be divided further in PD-1$^+$ Tregs and PD-1$^-$ effector cells [28]. FACS analysis of splenocytes, from 10-week-old mice, revealed that CD8$^+$ CD122$^+$ T cells from female NOD mice lack the expression of PD-1 (**Fig. 2A**), whereas CD8$^+$ CD122$^+$ T cells from 3-week-old NOD mice display similar expression of

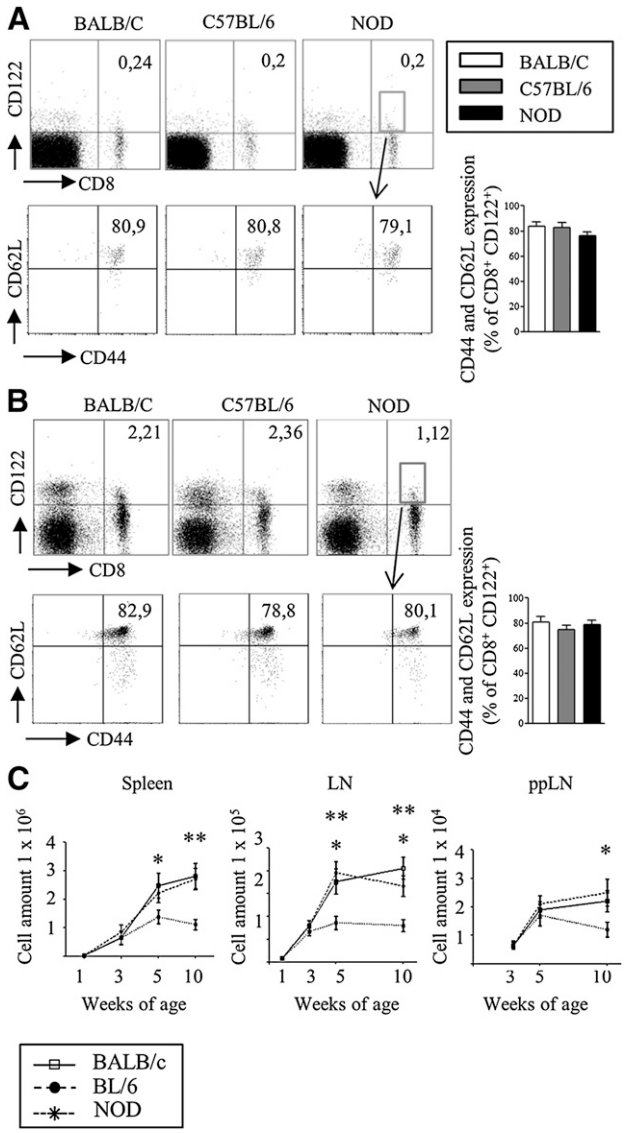


Figure 1. Reduction of CD8$^+$ CD122$^+$ T cells in 5- and 10-week-old NOD mice. Splenocytes from 1-week-old (A) or 10- to 12-week-old (B) Balb/c, BL/6, and NOD mice were stained with mAb for CD8, CD122, CD44, and CD62L and analyzed by flow cytometry. (A and B) Dot plots display representative profiles of CD8$^+$ CD122$^+$ cells (A and B, upper row) and the expression profile of CD44 and CD62L from CD8$^+$ CD122$^+$-gated cells (A and B, lower row) from 1-week-old (A) and 10- to 12-week-old (B) mice; numbers represent the percentages of cells in the indicated quadrant areas of total gated living lymphocytes. Bar graphs show the simultaneous expression of CD44 and CD62L inside of the CD8$^+$ CD122$^+$ population in 1-week-old (A) or 10- to 12-week-old (B) mice. At least 4 (A) or 5 (B) mice/group were analyzed. (C) Graphs display the absolute cell number of CD8$^+$ CD122$^+$ cells in the secondary lymphoid organs [spleen, lymph nodes (LN), and peripancreatic lymph nodes (ppLN)] in 1-, 3-, 5-, and 10- to 12-week-old Balb/c, BL/6, and NOD female mice. Statistical significance is indicated as follows: spleen 5-week-old mice, Balb/c vs. NOD ($*P < 0.05$), BL/6 vs. NOD ($*P < 0.05$), and spleen 10- to 12-week-old mice, Balb/c vs. NOD ($**P < 0.01$), BL/6 vs. NOD ($**P < 0.01$); lymph node 5-week-old mice, Balb/c vs. NOD ($*P < 0.05$), BL/6 vs. NOD ($**P < 0.01$), and lymph node 10- to 12-week-old mice, Balb/c vs. NOD ($**P < 0.01$), BL/6 vs. NOD ($*P < 0.05$); peripancreatic lymph node, 10- to 12-week-old mice, Balb/c vs. NOD ($*P < 0.05$), BL/6 vs. NOD ($*P < 0.05$). At least 4 mice/group were analyzed.

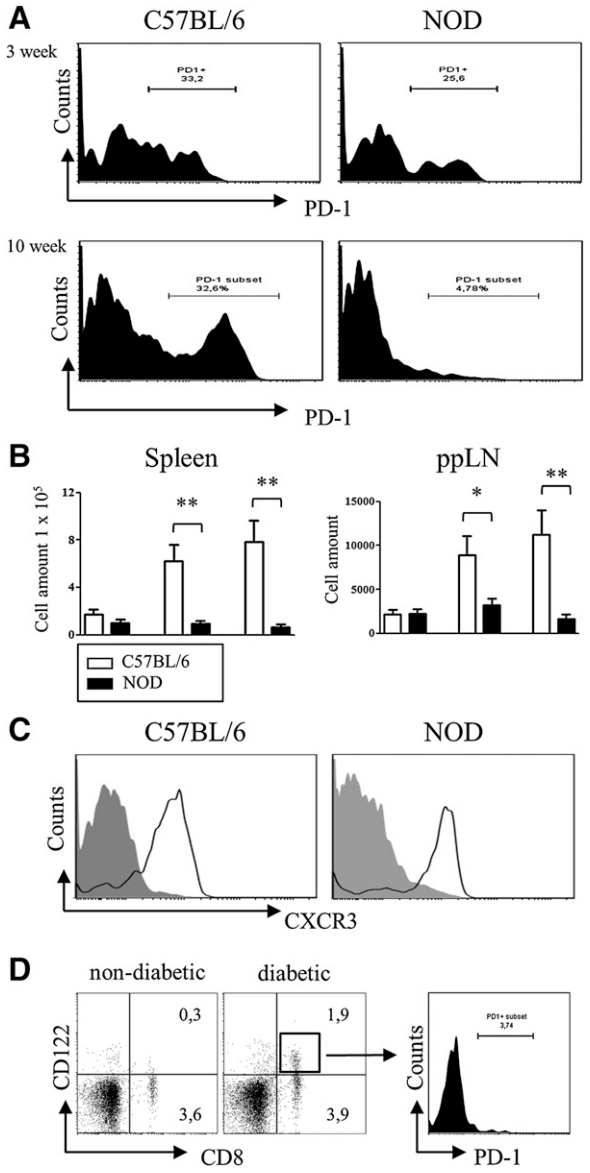


Figure 2. CD8$^+$ CD122$^+$ T cells in NOD mice lack PD-1 expression and are CXCR3$^+$. (A) Splenocytes from 3-week-old BL/6 and NOD mice (upper row) and from 10- to 12-week-old mice (lower row) were stained with mAb for CD8, CD122, and PD-1. The histograms display the expression of PD-1 in CD8$^+$ CD122$^+$-gated cells from BL/6 (left) or NOD (right) mice. (B) Bar graphs show the absolute cell number of CD8$^+$ CD122$^+$ PD-1$^+$ cells in the secondary lymphoid organs (spleen and peripancreatic lymph nodes) in 3-, 5-, and 10- to 12-week-old BL/6 (open bars) and NOD female (black bars) mice. Statistical significance is indicated; $*P < 0.05$; $**P < 0.01$. At least 4 mice/group were analyzed. (C) Splenocytes from 10- to 12-week-old BL/6 and NOD mice were stained with mAb for CD8, CD122, and CXCR3. Histograms show the expression of CXCR3 in CD8$^+$ CD122$^-$ (gray shaded) or CD8$^+$ CD122$^+$ (thick lines) cells from BL/6 (left) or NOD (right) mice. One representative mouse out of 5 is shown. (D) Pancreas cells isolated from nondiabetic female NOD mice (26 weeks old; left) and diabetic female NOD mice (middle) were stained for CD8, CD122, and PD-1. (Right) The filled, black histogram displays the expression of PD-1 on CD8$^+$ CD122$^+$-gated cells from diabetic female NOD mice (as indicated by the arrow from middle to right panels). One representative experiment of at least 3 is shown, and numbers represent percent cells in each quadrant.

PD-1 compared with the same cell population in 3-week-old BL/6 mice (Fig. 2A). Furthermore, we determined the total amount of CD8$^+$ CD122$^+$ PD-1$^+$ cells in 3-, 5-, and 10- to 12-week-old BL/6 and NOD mice. As shown in Fig. 2B, this cell population increases over age in the BL/6 mouse strain. However, we could not detect an increase of this cell population in NOD mice. Hence, almost all CD8$^+$ CD122$^+$ T cells in older NOD female mice are PD-1$^-$, which were described previously as effector cells [28]. In agreement with that, we found that almost all CD8$^+$ CD122$^+$ PD-1$^-$ cells express the chemokine receptor CXCR3 (Fig. 2C), which is also known to be expressed on activated/effector cells and is absent in resting naive cells [29, 30]. Thus, it seems that NOD female mice lack the regulatory CD8$^+$ CD122$^+$ PD-1$^+$ cell population. Given the fact that NOD females become diabetic earlier and at a greater frequency than NOD males, we wanted to investigate whether differences in this CD8$^+$ CD122$^+$ PD-1$^+$ cell population in NOD male or female mice were present. As shown in Supplemental Fig. 3A, NOD male mice possess the same amount of CD8$^+$ CD122$^+$ PD-1$^+$ cells compared with NOD female mice. Furthermore, we determined the total amount of CD8$^+$ CD122$^+$ PD-1$^+$ cells in NOD female mice that have not developed diabetes at the age of 26 weeks and compared them with diabetic NOD female mice. Again, we did not find any differences in the amount of these cells between these two groups (Supplemental Fig. 3B). However, when investigating pancreas- infiltrating T cells, we detected a significantly enhanced CD8$^+$ CD122$^+$ PD-1$^-$ cell population in the pancreas in diabetic NOD female mice compared with NOD female mice that have not developed diabetes at the age of 26 weeks (Fig. 2D).

With the consideration that CD8$^+$ CD122$^+$ T cells in NOD mice possess an effector cell phenotype (CD44$^+$ CD62L$^+$ CXCR3$^+$ PD-1$^-$), we wanted to test whether these CD8$^+$ CD122$^+$ T cells display effector function in vitro. To this end, CD8$^+$ CD122$^+$ and CD8$^+$ CD122$^-$ cells were purified and cultured in vitro after activation with CD3 and CD28. **Figure 3A** and **B** shows that CD8$^+$ CD122$^+$ T cells display a strongly enhanced proliferation capability compared with CD8$^+$ CD122$^-$ T cells. To exclude the possibility that an altered expression of CD3 or CD28 on CD8$^+$ CD122$^+$ or on CD8$^+$ CD122$^-$ cells contributes to these proliferation differences, expression levels of these 2 markers were analyzed by FACS. We found that CD8$^+$ CD122$^+$ and CD8$^+$ CD122$^-$ cells express the same level of CD3 and CD28 (Supplemental Fig. 4A). In agreement with other publications, we observed that CD8$^+$ CD122$^+$ T cells are responsible for the majority of IFN-γ production, whereas CD8$^+$ CD122$^-$ cells produce only small amounts of IFN-γ (Fig. 3C) [30, 31]. Moreover, we found that after activation, CD8$^+$ CD122$^+$ T cells produce a great amount of perforin and granzyme B (Fig. 3C).

Additionally, we checked for the regulatory capacity of CD8$^+$ CD122$^+$ T cells from NOD female mice. We used CD4$^+$CD25$^-$ cells as responder cells for the CD8$^+$CD122$^+$ suppression assay, as it is known that CD8$^+$ CD122$^+$ T cells from BL/6 mice regulate the CD4$^+$ cell [21, 32]. Figure 3D shows that CD8$^+$ CD122$^+$ T cells from NOD mice cannot inhibit the proliferation capability of CD4$^+$ T cells even when used in a 1:1 ratio. In contrast, we showed that CD8$^+$ CD122$^+$ T cells from BL/6 mice possess regulatory activity and suppress the proliferation capability of CD4$^+$ responder cells (Supplemental Fig. 4B). Hence, we conclude that CD8$^+$ CD122$^+$

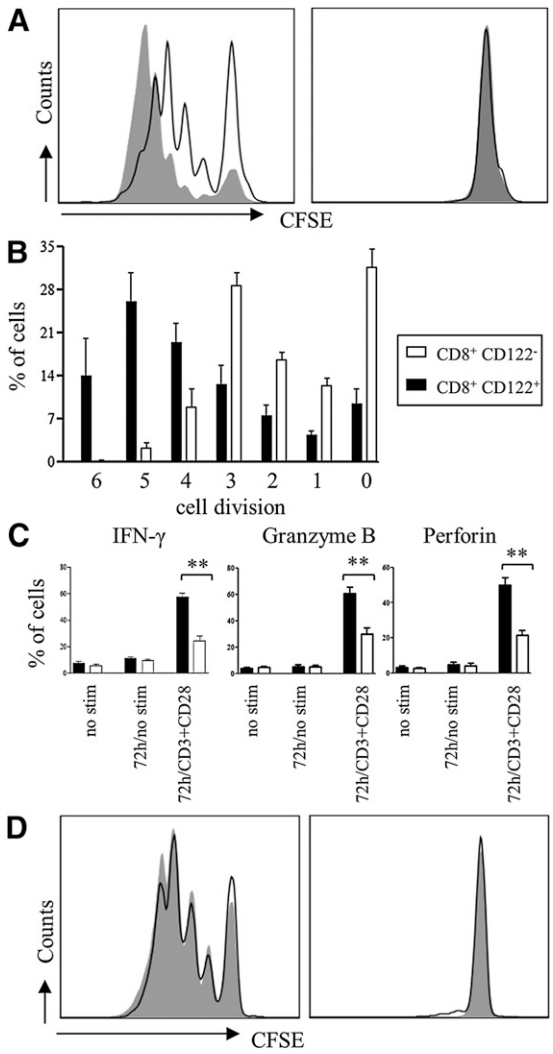


Figure 3. CD8+ CD122+ T cells possess effector function and lack regulatory activity. (A) Purified splenic CD8+ CD122− (open histograms) and CD8+ CD122+ (gray-filled histograms) cells from NOD mice were loaded with CFSE, subsequently stimulated with plate-bound CD3 (1 μg/ml) and soluble CD28 (5 μg/ml) mAb for 72 h, and analyzed by FACS. (Right) Equal loading of CFSE between CD8+ CD122− (open histogram) and CD8+ CD122+ (gray-filled histogram) cells. (Left) Proliferation of CD8+ CD122− and CD8+ CD122+ cells (CFSE dilution) after 72 h. (B) Bar graphs show semiquantitative measurement of proliferation capability of CD8+ CD122− and CD8+ CD122+ cells from 4 different experiments. Numbers on the *x*-axis indicate cell divisions. (C) IFN-γ, granzyme B, and perforin production of purified splenic CD8+ CD122− and CD8+ CD122+ cells were measured in vitro in unstimulated cells after 0 h (no stim) and after 72 h (72 h/no stim) and in cells stimulated with 1 μg/ml CD3 and 5 μg/ml CD28 mAb for 72 h in vitro. Statistical significance is indicated; $^{**}P < 0.01$. Bar graphs display the results of 4 individual experiments. (D) Suppression assay. CD4+ T cells (responder) from NOD mice were labeled with CFSE and subsequently stimulated with 1 μg/ml CD3 and 5 μg/ml CD28 mAb and cultured for 72 h (open histograms); proliferation was analyzed by FACS. In parallel, CD4+ T cells (responder) were activated in the same condition and cocultured with activated CD8+ CD122+ T cells (suppressor) in a 1:1 ratio (gray histograms). Proliferation of CD4+ T cells (responder) was determined by analyzing the CFSE profiles. The CFSE overlay shows the proliferation capability of CD4+ T cells (responder) cultured alone (open histograms) or in a 1:1 ratio with CD8+ CD122+ T cells (gray histograms). The CFSE assay was done in duplicate. The CFSE profiles shown are representative of 3 individual experiments.

T cells from NOD mice lack regulatory activity. These results indicate that the CD8+ CD122+ CXCR3+ PD-1− cells from NOD mice are indeed effector cells, at least in vitro.

Depletion of CD122+ CD8+ T cells delays the development of manifest hyperglycemia in NOD mice

In addition to the in vitro findings, we wanted to determine whether CD8+ CD122+ T cells also possess effector function in vivo. We depleted the remaining CD8+ CD122+ T cells in 6-week-old female NOD mice by i.v. injection of 50 μg anti-CD122 (clone TM-β1) or 50 μg isotype control (5 injections in intervals of 14 d). **Figure 4A** illustrates that 1 injection of 50 μg CD122 leads to complete depletion of CD8+ CD122+ cells. This effect was still seen after 14 d, when the second injection was performed (see Fig. 4A). Successful depletion of CD122+ cells was determined by FACS by use of anti-CD122 antibodies (clone 5H4) that recognize a different epitope than the TM-β1 clone to exclude epitope masking.

We found that anti-CD122 treatment prevented diabetes development in almost 60% versus 15% in mice treated with isotype mAb after a time period of 200 d ($P < 0.01$; Fig. 4B). These data were confirmed by histologic examination of those mice that remained free of diabetes at the end of the experiment. There was a marked reduction in the severity of insulitis in those mice that received anti-CD122 treatment (Fig. 4C). These data are in line with the hypothesis that CD8+ CD122+ T cells are indeed effector cells.

The depletion of CD122+ cells through the injection of anti-CD122 mAb may have side-effects, such as release of cytokines that could influence the status of the unaffected leukocytes or the proliferation of other lymphocyte subsets. Thus, we wanted to investigate whether the injection of CD122 mAb alters the peripheral lymphocyte pool in the NOD mice. Therefore, NOD mice were injected 5 times with CD122 mAb or an isotype control, as described above and killed after 200 d, and the peripheral lymphoid organs (spleen, lymph nodes, and peripancreatic lymph nodes) were analyzed by flow cytometry. We found that the depletion of CD122+ CD8+ T cells did not lead to an altered lymphocyte composition (not shown). Cell numbers, as well as the subpopulation, were normal in CD122-treated mice. Furthermore, as shown in **Fig. 5A**, CD122 treatment did not result in a reduction or enhancement of activated/memory CD4 or CD8 cells. In agreement with these data, we found that CD122 treatment did not change the activation status of CD4 or CD8 cells, as demonstrated by comparable expression of activation markers (CD69, CD25, or CD44) compared with the isotype control (Fig. 5A). Next, we investigated whether the depletion of CD122+ cells could influence the CD4+ CD25+ Foxp3+ population. Again, treatment with CD122 had no impact on this cell population. Figure 5B shows that the amount of Foxp3+ CD4 Tregs was normal in mice that were treated with CD122 mAb. These results exclude the possibility that CD122 treatment leads to an expansion of Foxp3+ Tregs that could delay the diabetes progression.

CD8+ CD122+ T cells promote diabetes progression

Insulin-dependent autoimmune diabetes can be transferred from diabetic NOD mice to NOD SCID mice through the injection of splenocytes. We wanted to know whether the depletion of CD122+

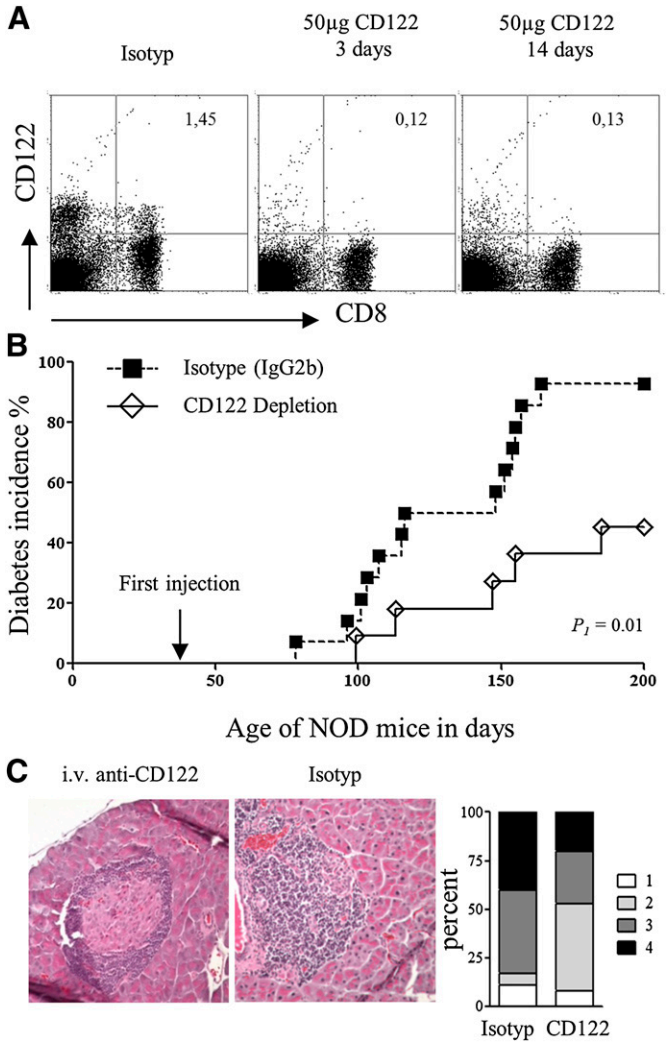


Figure 4. Treatment with CD122 mAb delays and reduces the diabetes development in NOD mice. (A) NOD female mice were injected with 50 μg anti-CD122 i.v. mAb or with the isotype control. Three or 14 d after injection, mice were killed, and splenocytes were analyzed for $CD122^+$ cells by flow cytometry. Numbers represent the percentages of cells in the indicated quadrant areas of total gated living lymphocytes. (B) Six-week-old NOD female mice were injected with 50 μg anti-CD122 mAb (11 mice) or with 50 μg anti-IgG2b (isotype; 14 mice), and diabetes incidence was measured. The day of the first injection is marked by an arrow; P_1, CD122 treatment versus isotype. (C, left and middle) H&E staining. Histogram sections of the pancreas from 1 representative mouse from each group that was diabetes free after 200 d. (Right) Mononuclear infiltration was scored in pancreatic islets from isotype- and CD122-treated NOD mice after 200 d of diabetes-free mice.

cells in diabetic NOD mice before the cell transfer results in a delay of diabetes development in recipient NOD SCID mice. The transfer of splenocytes obtained from diabetic NOD mice treated with isotype antibody results in diabetes development in all NOD SCID recipients within 16 weeks after transfer (**Fig. 6**). In contrast, NOD SCID recipients receiving splenocytes from anti-CD122 mAb-treated mice displayed a delayed diabetes progression. Interestingly, almost 45% of recipient mice remain diabetes free even at d 180 after splenocyte transfer. To ensure that the transfer of cells was successful in those mice that remained free of diabetes, the secondary lymphatic organs were investigated for the presence of donor splenocytes, 180 d after cell transfer. We found that all NOD SCID mice possessed donor lymphocytes in the spleen, indicating that the lack of diabetes development was not a result of a failure in the cell transfer (not shown).

$CD8^+$ $CD122^+$ T cells accelerate the development of diabetes when cotransferred with diabetogenic splenocytes in NOD SCID mice

To confirm further that $CD8^+$ $CD122^+$ T cells are important players in the progression of autoimmune diabetes, we investigated whether the cotransfer of in vitro-activated $CD8^+$ $CD122^+$ cells could accelerate diabetes development after cell transfer in NOD SCID recipients. As shown in **Fig. 7**, the cotransfer of 1×10^6 in vitro–activated $CD8^+$ $CD122^+$ cells isolated from nondiabetic NOD mice, together with 5×10^6 splenocytes from diabetic NOD mice, significantly accelerates the onset of diabetes in the NOD SCID recipient mice, compared with NOD SCID mice that received same number of splenocytes together with in vitro–activated $CD8^+$ $CD122^-$ cells ($P < 0.01$). These data suggest that $CD8^+$ $CD122^+$ T cells are involved in the acceleration of diabetes development in the NOD mice.

DISCUSSION

In this study, we describe for the first time that the $CD8^+$ $CD122^+$ $CXCR3^+$ $PD\text{-}1^-$ cell population is strongly involved in the development of diabetes in NOD mice. We demonstrated that depletion of these cells delays progression to overt diabetes in NOD mice, whereas the cotransfer of activated $CD8^+$ $CD122^+$ $CXCR3^+$ $PD\text{-}1^-$ with splenocytes from diabetic donors into NOD SCID mice accelerates diabetes development in recipient mice. Moreover, we demonstrate here that NOD mice lack the recently described regulatory $CD8^+$ $CD122^+$ $PD\text{-}1^+$ cell population.

By investigating the $CD8^+$ $CD122^+$ T cell pool in NOD mice and diabetes-resistant mouse strains, we found that 10- to 12-week-old NOD mice have a marked reduction in the absolute number of $CD8^+$ $CD122^+$ T cells in secondary lymphoid organs. This is a result of an absolute reduction of the regulatory $CD8^+$ $CD122^+$ $PD\text{-}1^+$ cell population, whereas the $CD8^+$ $CD122^+$ $PD\text{-}1^-$ cell population is only minimal affected. Interestingly, we found comparable numbers of $CD8^+$ $CD122^+$ $PD\text{-}1^+$ and $CD8^+$ $CD122^+$ $PD\text{-}1^-$ T cells when investing 1- and 3-week-old NOD and diabetes-resistant mouse strains. Furthermore, we could not detect any differences in the thymic $CD8^+$ $CD122^+$ cell population of young and older mice (data not shown). These results indicate that the decrease of $CD8^+$ $CD122^+$ $PD\text{-}1^+$ Tregs occurs during aging and is most likely not a developmental defect. Furthermore, it seems that this cell population from NOD mice has no surviving defect, as shown by ex vivo staining with Annexin V. One possible explanation is that these cells have disadvantages in steady-state proliferation. It would be of particular interest to measure the steady-state proliferation of these cells in the future. It is not clear from our results whether the loss of the $CD8^+$ $CD122^+$ $PD\text{-}1^+$ cell population contributes to

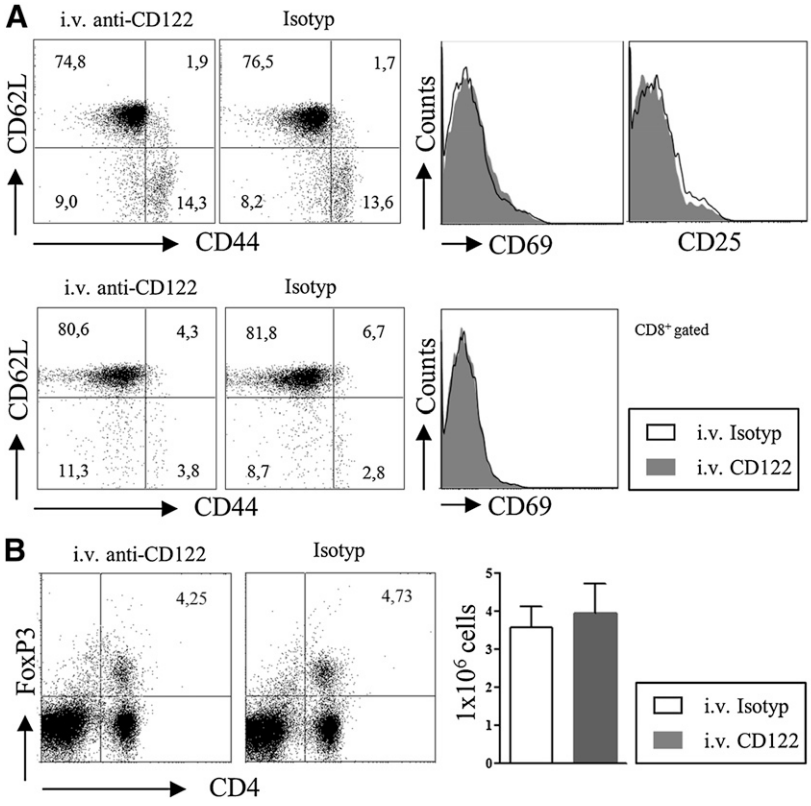


Figure 5. Treatment with CD122 mAb does not alter lymphocyte composition within the spleen. NOD female mice were treated i.v., 5 times with 50 μg CD122 or with the isotype control. After 200 d, splenocytes from CD122-treated or control mice were analyzed by flow cytometry. (A) CD4$^+$ (upper) or CD8$^+$ (lower) splenocytes were gated and analyzed for the expression of the activation markers CD25, CD44, CD62L, and CD69. Histogram overlays compare the expression of CD25 and CD69 on CD4 cells (upper) or CD69 expression on CD8 cells (lower) between CD122-treated (gray-filled histograms) or control (open histograms) mice. At least 5 mice/group were analyzed. (B) Numbers represent the percentages of cells in the indicated quadrant. Mice were analyzed for Foxp3 expression. Bar graphs show the absolute number of Tregs defined as CD4$^+$ Foxp3$^+$. At least 3 mice/group were analyzed.

the autoimmunity, especially in relation to the development of diabetes observed in NOD mice. However, this seems more unlikely, as we found that NOD male mice and NOD female mice that do not become diabetic until the age of 26 weeks also lack the CD8$^+$ CD122$^+$ PD-1$^+$ regulatory cell population. Further experiments are necessary to define the role of this cell population during the development of diabetes.

CD8$^+$ CD122$^+$ CXCR3$^+$ PD-1$^-$ T cells from NOD mice, as well as from diabetic-resistant mouse strains, express on their surface CD44 and CD62L and therefore, resemble central memory or effector cells. Our in vitro studies clearly showed that these CD8$^+$ CD122$^+$ cells from NOD mice possess effector function. In contrast to other studies, where it has been shown that CD8$^+$ CD122$^+$ cells from BL/6 mice possess regulatory function [21, 23], we found that CD8$^+$ CD122$^+$ cells from NOD mice lack regulatory function, as they are not able to suppress the proliferation capability of CD4$^+$ cells. This observation is most likely a result of the lack of the regulatory PD-1$^+$ cell population

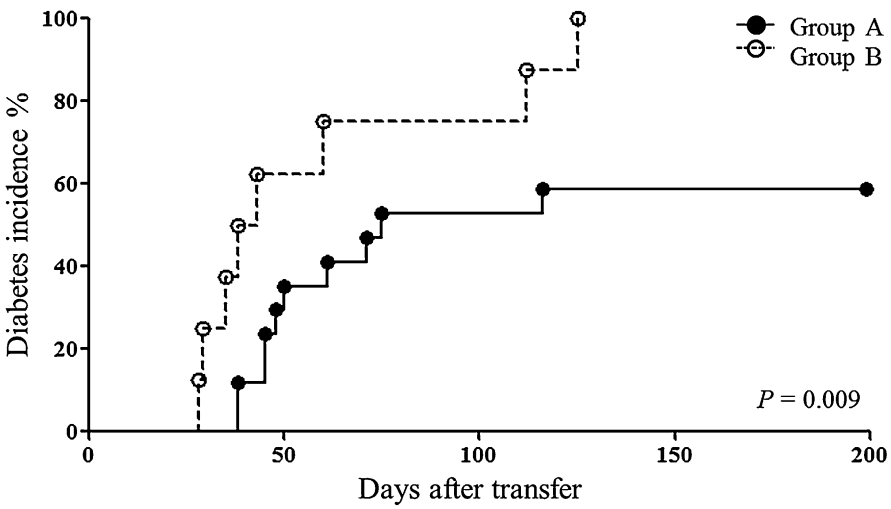


Figure 6. Depletion of CD122$^+$ cells before cell transfer delayed diabetes development in NOD SCID recipient mice. Three days before cell transfer of diabetogenic splenocytes from NOD female mice (donors) into NOD SCID recipient mice, the donor mice were treated with mAb against CD122 ($n = 16$) or with the isotype control ($n = 8$). Those recipient mice who received the CD122-depleted splenocytes from the NOD donors were termed Group A, whereas those who received the splenocytes from the NOD donors that were only treated with the isotype control were termed Group B. Graph displays the incidence of diabetes in NOD SCID mice in days after transfer.

within the CD8$^+$ CD122$^+$ cell pool of NOD mice. In agreement with this, IL-10, which has been reported to mediate the regulating capacity of CD8$^+$ CD122$^+$ cells, could not be detected in a significant amount [33]. However, it has already been shown that CD8$^+$ CD122$^+$ T cells in NOD mice could have a regulatory function. Shameli et al. [34] described a CD8$^+$ CD122$^+$ population that arose spontaneously in 17.4α/8.3β TCR-transgenic NOD mice, and they were able to clearly display that this cell population has regulatory functions and was responsible for the resistance of these mice to developing autoimmune diabetes. They also showed that this cell population resemble our CD8$^+$ CD122$^+$ cells which we described here (they also express CD44, CD62L and CXCR3). However, Shameli et al. [34] did not investigate whether these cells express PD-1. In addition, it is important to note that this cell population only arose in 17.4α/8.3β TCR-transgenic NOD mice that express a low-affinity TCR for IGRP$_{206–214}$. NOD mice expressing a high-affinity TCR for IGRP$_{206–214}$ lack this cell population, as do "normal NOD" mice [35]. More importantly, Shameli et al. [34] showed that these cells were anergic in response to antigenic stimulation ex vivo, in contrast to the CD8$^+$ CD122$^+$ cells that we described here. Therefore, it seems that these cell populations described here and by Shameli et al. [34] share similarities in the expression profile but are distinct in function. More strikingly, we could demonstrate that the CD8$^+$ CD122$^+$ PD-1$^-$ cell population displayed effector function and lacked regulatory activity in vivo, which further strengthens our hypothesis that these cells from NOD mice are primarily effector cells. We could also demonstrate that NOD female mice treated with CD122 mAb displayed protection against diabetes development. However, as shown in Fig. 4A, beside CD8$^+$ cells, NK cells also express CD122 on their surface. Therefore, treatment of NOD mice with anti-CD122 mAb (clone TM-β1) leads also to a depletion of NK cells (Fig. 4A). To our knowledge, NK cells from NOD mice do not express a marker that can be targeted by currently available antibodies for depletion, such as NK1.1. Thus, we cannot prove directly that the depletion of the CD122$^+$ NK cells protected the NOD mice from diabetes development. However, it has been shown recently that depletion of NK cells in the transgenic NK1.1 NOD mouse has no influence on the spontaneous diabetes development in these mice [36].

Given the fact that the transfer of highly purified CD8$^+$ CD122$^+$ cells accelerates diabetes development, it seems more likely that the depletion of the CD8$^+$ CD122$^+$ cell population is responsible for protection against diabetes development rather than the depletion of CD122$^+$ NK cells.

How CD8$^+$ CD122$^+$ CXCR3$^+$ PD-1$^-$ cells mediate the progression of diabetes development still remains elusive. Interestingly, in agreement with recently published results, we found that in naive mice, 85% of CXCR3$^+$ cells were CD8$^+$ CD122$^+$, indicating that the expression of this receptor is relatively restricted to this cell population [30]. This is in line with the idea that CXCR3 is expressed in effector cells and is absent in naive T cells; i.e., we have shown that our CD8$^+$ CD122$^+$ CXCR3$^+$ T cells display effector function. It is important to note that the depletion of CD122$^+$ T cells through the injection of mAb against CD122 also leads to a dramatic decrease of CXCR3$^+$ cells (not shown). CXCR3 is a chemokine receptor that plays an important role in T cell trafficking and function. CXCR3 binds 3 chemokines, namely CXCL9, CXCL10, and CXCL11; these induce migration of activated T cells in vitro and at in vivo sites of inflammation [37–40].

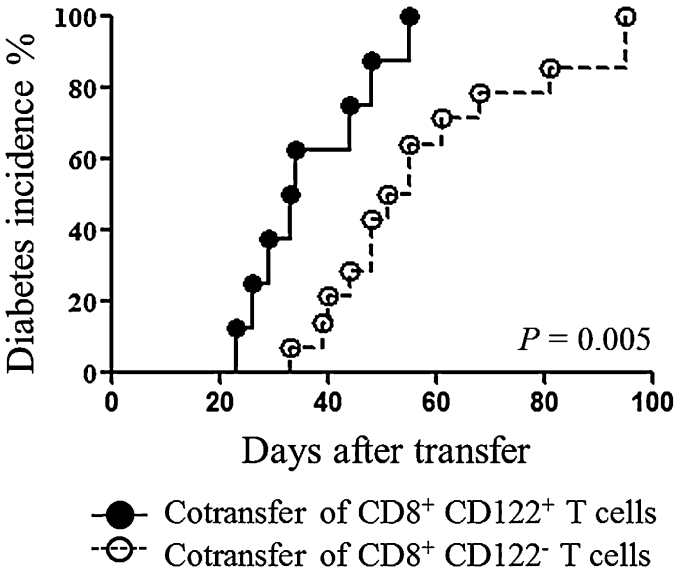


Figure 7. Transfer of activated CD8$^+$ CD122$^+$ T cells into NOD SCID mice accelerate diabetes development. Cotransfer of purified and in vitro-activated 1 × 10^6 CD8$^+$ CD122$^+$ T cells (n = 8) or 1 × 10^6 CD8$^+$ CD122$^-$ T cells (n = 11), together with 5 × 10^6 splenocytes from diabetogenic NOD mice into NOD SCID recipient mice. Graph displays the incidence of diabetes in NOD SCID mice during follow-up.

Frigerio et al. [41] could recently demonstrate that during insulitis, the β-cell itself produces CXCL9 and CXCL10 in response to IFN-γ. Subsequently, CXCL9 and CXCL10 recruit CXCR3$^+$ effector T cells to the sites of insulitis. Another study also showed that β cell–specific expression of CXCL10 causes infiltration of CD8$^+$ T cells into the Langerhans islets [42]. In these studies, CXCR3$^+$ T cells were not characterized further, but given the fact that most of the CXCR3$^+$ cells are also CD8$^+$ CD122$^+$, it seems possible that the cell population characterized here was responsible for the infiltration of the Langerhans islets in these studies. More strikingly, in CXCR3$^{-/-}$ mice, the onset of T1D is delayed substantially [41], similar to what we observed when CD122$^+$ cells are depleted. It seems possible that the insulitis process reflects an inflammatory loop caused by T cell–derived IFN-γ, which, in turn, leads to expression of CXCL9 and CXCL10 by β cells, allowing the migration of CXCR3$^+$ CD122$^+$ CD8$^+$ T cells into the Langerhans islets. Again, it is important to note that we and other groups were able to demonstrate that CD8$^+$ CD122$^+$ T cells are the main source of T cell–derived IFN-γ. More interestingly, we also demonstrated that NOD female mice that become diabetic display an infiltration of CD8$^+$ CD122$^+$ PD-1$^-$ cells inside of the pancreas, whereas in nondiabetic NOD female mice, this cell population was hardly detectable. However, the initial trigger that initiates this process still remains elusive. Tanaka et al. [43] demonstrated recently that a viral infection of the pancreas leads to simultaneous expression of IFN-γ and CXCL10 within the Langerhans islets and subsequently, to a massive infiltration of CXCR3$^+$ T cells.

The present findings regarding the role of CD8$^+$ CD122$^+$ CXCR3$^+$ T cells in the destruction of islet endocrine cells provide new insights into strategies for prevention of T1D. Development of antagonists and neutralizing agents for the IFN-γ–induced CXCL10/CXCR3 axis may represent 1 therapeutic option.

AUTHORSHIP

B.A. performed the statistical analysis, carried out the FACS analysis, performed the proliferation and suppression assays, conceived of and coordinated the study, and drafted the manuscript. L.W. carried out the adoptive-transfer experiments and performed the FACS analysis, histological examination, and cell separation. J.E. participated during the cell separation. J.S. participated in the design of the study and helped to draft the manuscript. All authors read and approved the final manuscript.

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DISCLOSURES

The authors have no financial conflicts of interest.

REFERENCES

1. Atkinson, M. A., Leiter, E. H. (1999) The NOD mouse model of type 1 diabetes: as good as it gets? *Nat. Med.* **5,** 601–604.
2. Anderson, M. S., Bluestone, J. A. (2005) The NOD mouse: a model of immune dysregulation. *Annu. Rev. Immunol.* **23,** 447–485.
3. Wong, F. S., Visintin, I., Wen, L., Flavell, R. A., Janeway, Jr., C. A. (1996) CD8 T cell clones from young nonobese diabetic (NOD) islets can transfer rapid onset of diabetes in NOD mice in the absence of CD4 cells. *J. Exp. Med.* **183,** 67–76.
4. Brodie, G. M., Wallberg, M., Santamaria, P., Wong, F. S., Green, E. A. (2008) B-Cells promote intra-islet CD8+ cytotoxic T-cell survival to enhance type 1 diabetes. *Diabetes* **57,** 909–917.
5. DiLorenzo, T. P., Graser, R. T., Ono, T., Christianson, G. J., Chapman, H. D., Roopenian, D. C., Nathenson, S. G., Serreze, D. V. (1998) Major histocompatibility complex class I-restricted T cells are required for all but the end stages of diabetes development in nonobese diabetic mice and use a prevalent T cell receptor alpha chain gene rearrangement. *Proc. Natl. Acad. Sci. USA* **95,** 12538–12543.
6. Emamaullee, J. A., Davis, J., Merani, S., Toso, C., Elliott, J. F., Thiesen, A., Shapiro, A. M. (2009) Inhibition of Th17 cells regulates autoimmune diabetes in NOD mice. *Diabetes* **58,** 1302–1311.
7. Grinberg-Bleyer, Y., Baeyens, A., You, S., Elhage, R., Fourcade, G., Gregoire, S., Cagnard, N., Carpentier, W., Tang, Q., Bluestone, J., Chatenoud, L., Klatzmann, D., Salomon, B. L., Piaggio, E. (2010) IL-2 reverses established type 1 diabetes in NOD mice by a local effect on pancreatic regulatory T cells. *J. Exp. Med.* **207,** 1871–1878.
8. Tang, Q., Adams, J. Y., Penaranda, C., Melli, K., Piaggio, E., Sgouroudis, E., Piccirillo, C. A., Salomon, B. L., Bluestone, J. A. (2008) Central role of defective interleukin-2 production in the triggering of islet autoimmune destruction. *Immunity* **28,** 687–697.
9. Bendelac, A., Carnaud, C., Boitard, C., Bach, J. F. (1987) Syngeneic transfer of autoimmune diabetes from diabetic NOD mice to healthy neonates. Requirement for both L3T4+ and Lyt-2+ T cells. *J. Exp. Med.* **166,** 823–832.
10. Bedossa, P., Bendelac, A., Bach, J. F., Carnaud, C. (1989) Syngeneic T cell transfer of diabetes into NOD newborn mice: in situ studies of the autoimmune steps leading to insulin-producing cell destruction. *Eur. J. Immunol.* **19,** 1947–1951.
11. Miller, B. J., Appel, M. C., O'Neil, J. J., Wicker, L. S. (1988) Both the Lyt-2+ and L3T4+ T cell subsets are required for the transfer of diabetes in nonobese diabetic mice. *J. Immunol.* **140,** 52–58.
12. Varey, A. M., Hutchings, P., O'Reilly, L., Hussell, T., Waldmann, H., Simpson, E., Cooke, A. (1991) The development of insulin-dependent diabetes mellitus in non-obese diabetic mice: the role of CD4+ and CD8+ T cells. *Biochem. Soc. Trans.* **19,** 187–191.
13. Peterson, J. D., Haskins, K. (1996) Transfer of diabetes in the NOD-scid mouse by CD4 T-cell clones. Differential requirement for CD8 T-cells. *Diabetes* **45,** 328–336.
14. Phillips, J. M., Haskins, K., Cooke, A. (2005) MAdCAM-1 is needed for diabetes development mediated by the T cell clone, BDC-2.5. *Immunology* **116,** 525–531.
15. Wang, B., Gonzalez, A., Benoist, C., Mathis, D. (1996) The role of CD8+ T cells in the initiation of insulin-dependent diabetes mellitus. *Eur. J. Immunol.* **26,** 1762–1769.
16. Walzer, T., Arpin, C., Beloeil, L., Marvel, J. (2002) Differential in vivo persistence of two subsets of memory phenotype CD8 T cells defined by CD44 and CD122 expression levels. *J. Immunol.* **168,** 2704–2711.
17. Zhang, X., Sun, S., Hwang, I., Tough, D. F., Sprent, J. (1998) Potent and selective stimulation of memory-phenotype CD8+ T cells in vivo by IL-15. *Immunity* **8,** 591–599.
18. Judge, A. D., Zhang, X., Fujii, H., Surh, C. D., Sprent, J. (2002) Interleukin 15 controls both proliferation and survival of a subset of memory-phenotype CD8(+) T cells. *J. Exp. Med.* **196,** 935–946.
19. Takayama, E., Seki, S., Ohkawa, T., Ami, K., Habu, Y., Yamaguchi, T., Tadakuma, T., Hiraide, H. (2000) Mouse CD8+ CD122+ T cells with intermediate TCR increasing with age provide a source of early IFN-gamma production. *J. Immunol.* **164,** 5652–5658.
20. Motegi, A., Kinoshita, M., Inatsu, A., Habu, Y., Saitoh, D., Seki, S. (2008) IL-15-induced CD8+CD122+ T cells increase antibacterial and anti-tumor immune responses: implications for immune function in aged mice. *J. Leukoc. Biol.* **84,** 1047–1056.
21. Rifa'i, M., Kawamoto, Y., Nakashima, I., Suzuki, H. (2004) Essential roles of CD8+CD122+ regulatory T cells in the maintenance of T cell homeostasis. *J. Exp. Med.* **200,** 1123–1134.
22. Saitoh, O., Abiru, N., Nakahara, M., Nagayama, Y. (2007) CD8+CD122+ T cells, a newly identified regulatory T subset, negatively regulate Graves' hyperthyroidism in a murine model. *Endocrinology* **148,** 6040–6046.
23. Endharti, A. T., Okuno, Y., Shi, Z., Misawa, N., Toyokuni, S., Ito, M., Isobe, K., Suzuki, H. (2011) CD8+CD122+ regulatory T cells (Tregs) and CD4+ Tregs cooperatively prevent and cure CD4+ cell-induced colitis. *J. Immunol.* **186,** 41–52.
24. Arndt, B., Kalinski, T., Reinhold, D., Thielitz, A., Roessner, A., Schraven, B., Simeoni, L. (2013) Cooperative immunoregulatory function of the transmembrane adaptor proteins SIT and LAX. *J. Leukoc. Biol.* **93,** 353–362.
25. Arndt, B., Poltorak, M., Kowtharapu, B. S., Reichardt, P., Philipsen, L., Lindquist, J. A., Schraven, B., Simeoni, L. (2013) Analysis of TCR activation kinetics in primary human T cells upon focal or soluble stimulation. *J. Immunol. Methods* **387,** 276–283.
26. Ohly, P., Dohle, C., Abel, J., Seissler, J., Gleichmann, H. (2000) Zinc sulphate induces metallothionein in pancreatic islets of mice and protects against diabetes induced by multiple low doses of streptozotocin. *Diabetologia* **43,** 1020–1030.
27. Berzins, S. P., Venanzi, E. S., Benoist, C., Mathis, D. (2003) T-Cell compartments of prediabetic NOD mice. *Diabetes* **52,** 327–334.
28. Dai, H., Wan, N., Zhang, S., Moore, Y., Wan, F., Dai, Z. (2010) Cutting edge: programmed death-1 defines CD8+CD122+ T cells as regulatory versus memory T cells. *J. Immunol.* **185,** 803–807.
29. Groom, J. R., Luster, A. D. (2011) CXCR3 ligands: redundant, collaborative and antagonistic functions. *Immunol. Cell Biol.* **89,** 207–215.
30. Oghumu, S., Dong, R., Varikuti, S., Shawler, T., Kampfrath, T., Terrazas, C. A., Lezama-Davila, C., Ahmer, B. M., Whitacre, C. C., Rajagopalan, S., Locksley, R., Sharpe, A. H., Satoskar, A. R. (2013) Distinct populations of innate CD8+ T cells revealed in a CXCR3 reporter mouse. *J. Immunol.* **190,** 2229–2240.
31. Sato, K., Kinoshita, M., Motegi, A., Habu, Y., Takayama, E., Nonoyama, S., Hiraide, H., Seki, S. (2005) Critical role of the liver CD8+ CD122+ T cells in the generalized Shwartzman reaction of mice. *Eur. J. Immunol.* **35,** 593–602.

32. Rifa'i, M., Shi, Z., Zhang, S. Y., Lee, Y. H., Shiku, H., Isobe, K., Suzuki, H. (2008) CD8+CD122+ regulatory T cells recognize activated T cells via conventional MHC class I-alphabetaTCR interaction and become IL-10-producing active regulatory cells. *Int. Immunol.* **20,** 937–947.
33. Endharti, A. T., Rifa'I, M., Shi, Z., Fukuoka, Y., Nakahara, Y., Kawamoto, Y., Takeda, K., Isobe, K., Suzuki, H. (2005) Cutting edge: CD8+CD122+ regulatory T cells produce IL-10 to suppress IFN-gamma production and proliferation of CD8+ T cells. *J. Immunol.* **175,** 7093–7097.
34. Shameli, A., Yamanouchi, J., Tsai, S., Yang, Y., Clemente-Casares, X., Moore, A., Serra, P., Santamaria, P. (2013) IL-2 promotes the function of memory-like autoregulatory CD8+ T cells but suppresses their development via FoxP3+ Treg cells. *Eur. J. Immunol.* **43,** 394–403.
35. Yamanouchi, J., Rainbow, D., Serra, P., Howlett, S., Hunter, K., Garner, V. E., Gonzalez-Munoz, A., Clark, J., Veijola, R., Cubbon, R., Chen, S. L., Rosa, R., Cumiskey, A. M., Serreze, D. V., Gregory, S., Rogers, J., Lyons, P. A., Healy, B., Smink, L. J., Todd, J. A., Peterson, L. B., Wicker, L. S., Santamaria, P. (2007) Interleukin-2 gene variation impairs regulatory T cell function and causes autoimmunity. *Nat. Genet.* **39,** 329–337.
36. Beilke, J. N., Meagher, C. T., Hosiawa, K., Champsaur, M., Bluestone, J. A., Lanier, L. L. (2012) NK cells are not required for spontaneous autoimmune diabetes in NOD mice. *PLoS ONE* **7,** e36011.
37. Campanella, G. S., Grimm, J., Manice, L. A., Colvin, R. A., Medoff, B. D., Wojtkiewicz, G. R., Weissleder, R., Luster, A. D. (2006) Oligomerization of CXCL10 is necessary for endothelial cell presentation and in vivo activity. *J. Immunol.* **177,** 6991–6998.
38. Cole, K. E., Strick, C. A., Paradis, T. J., Ogborne, K. T., Loetscher, M., Gladue, R. P., Lin, W., Boyd, J. G., Moser, B., Wood, D. E., Sahagan, B. G., Neote, K. (1998) Interferon-inducible T cell alpha chemoattractant (I-TAC): a novel non-ELR CXC chemokine with potent activity on activated T cells through selective high affinity binding to CXCR3. *J. Exp. Med.* **187,** 2009–2021.
39. Loetscher, M., Loetscher, P., Brass, N., Meese, E., Moser, B. (1998) Lymphocyte-specific chemokine receptor CXCR3: regulation, chemokine binding and gene localization. *Eur. J. Immunol.* **28,** 3696–3705.
40. Lu, B., Humbles, A., Bota, D., Gerard, C., Moser, B., Soler, D., Luster, A. D., Gerard, N. P. (1999) Structure and function of the murine chemokine receptor CXCR3. *Eur. J. Immunol.* **29,** 3804–3812.
41. Frigerio, S., Junt, T., Lu, B., Gerard, C., Zumsteg, U., Holländer, G. A., Piali, L. (2002) Beta cells are responsible for CXCR3-mediated T cell infiltration in insulitis. *Nat. Med.* **8,** 1414–1420.
42. Rhode, A., Pauza, M. E., Barral, A. M., Rodrigo, E., Oldstone, M. B., von Herrath, M. G., Christen, U. (2005) Islet-specific expression of CXCL10 causes spontaneous islet infiltration and accelerates diabetes development. *J. Immunol.* **175,** 3516–3524.
43. Tanaka, S., Nishida, Y., Aida, K., Maruyama, T., Shimada, A., Suzuki, M., Shimura, H., Takizawa, S., Takahashi, M., Akiyama, D., Arai-Yamashita, S., Furuya, F., Kawaguchi, A., Kaneshige, M., Katoh, R., Endo, T., Kobayashi, T. (2009) Enterovirus infection, CXC chemokine ligand 10 (CXCL10), and CXCR3 circuit: a mechanism of accelerated beta-cell failure in fulminant type 1 diabetes. *Diabetes* **58,** 2285–2291.

KEY WORDS:
T cells · insulitis · autoimmunity · inflammation