Lymph Node T-Cells Do Not Optimally Transfer Diabetes in NOD Mice

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The nonobese diabetic mouse is a model of spontaneous development of autoimmune type I diabetes. The disease can be induced in young, irradiated recipients by injecting splenic T-cells from diabetic donors. The adoptive transfer of diabetes requires the presence of both $CD4^+$ and $CD8^+$ splenic T-cell subsets. To test whether diabetogenic cells distribute in other lymphoid organs of diabetic mice, we first analyzed lymph node cells. Lymph node cells were much less efficient in transferring diabetes than splenocytes. This inefficacious transfer was not attributable to the absence of hematopoietic precursors or a lack of macrophages. Lymph node cells did not protect from the transfer of diabetes by splenocytes, indicating the absence of suppressor cells. Although $CD8^+$ lymph node T-cells seemed functionally comparable to $CD8^+$ splenocytes, $CD4^+$ lymph node T-cells failed to cooperate with $CD8^+$ splenocytes to transfer diabetes. Our study suggests that diabetogenic cells are not evenly distributed in the different lymphoid organs. This may reflect a differential migration pattern of pathogenic T-cells in this animal model. *Diabetes* 42:1823–28, 1993

The NOD mouse spontaneously develops type I diabetes and provides a valid experimental model for the human disease (1). NOD mice of both sexes show lymphocytic infiltration in the islets of Langerhans (insulitis) by 4–6 wk of age (2), but the clinical disease is observed after 12 wk of age predominantly in females (1). The involvement of T-cells in the destruction of insulin-secreting β-cells of the islets of Langerhans has been abundantly documented. Neonatal thymectomy (3), anti-T-cell monoclonal antibody (4–6), and cyclosporin A (7) prevent the disease. It is possible to induce diabetes by using adoptive transfer of spleen cells from diabetic donors into young, irradiated animals (8) or into neonates (9).

Both $CD4^+$ and $CD8^+$ T-cells are required for the transfer of diabetes in prediabetic NOD recipients (9–11); however, the respective role of each subset remains controversial. A complex network of regulatory circuits controls the development and function of diabetes effector cells. Suppressor, helper, and cytotoxic cells were reported to be either $CD4^+$ or $CD8^+$ cells (12–16). Macrophages most likely exert regulatory functions (13,17,18). Several lines of evidence indicate that $CD4^+$ and $CD8^+$ cells interact differently with β-cells at different stages in T-cell–mediated β-cell destruction. In particular, various studies suggest that $CD4^+$ cells are the helper cells for the activation of $CD8^+$ effector cells (11,15). However, Bradley et al. (14) showed that islet-specific cloned $CD4^+$ T-cells could initiate islet destruction in the absence of detectable $CD8^+$ T-cells. In addition, Christianson et al. (19) reported that although $CD4^+$ T-cells from diabetic donor spleens were capable of mediating the disease in the absence of demonstrable $CD8^+$ T-cells, the presence of this latter cell type strongly accelerated the pathogenic processes.

Adoptive transfer of diabetes demonstrates the presence of diabetogenic T-cells in the spleen of diabetic mice. Whether diabetogenic cells are evenly distributed throughout the lymphoid organs or localized specifically in particular organs has not yet been investigated. To address this question, we first considered the lymph nodes as a source of pathogenic cells. We show that diabetes is poorly transferred via LN cells. This feature is not attributable to the presence of suppressor cells or to the absence of nonlymphoid cells, which are found in the spleen. LN $CD4^+$ cells appear to differ from the spleen

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Type I diabetes, insulin-dependent diabetes mellitus; LN cells, lymph node cells; IgG, immunoglobulin G.

$CD4^+$ subset in that they fail to optimally transfer diabetes with spleen $CD8^+$ cells.

RESEARCH DESIGN AND METHODS

NOD mice were bred in our own facilities under specific pathogen-free conditions. Recipients were males, 7–10 wk of age, irradiated with 7.5 Gy the day before cell transfer. Female mice, newly diagnosed with diabetes or 4–6 wk of age, were used as donors. Thymectomy was performed under ether anesthesia by suction with a Pasteur pipet 2 wk before transfer.

Cell preparation and fractionation. LN cells were isolated from a pool of mesenteric and peripheral (inguinal and axillary) lymph nodes or from a pool of pancreatic lymph nodes. Single-cell suspensions of spleen and LN cells were prepared in RPMI-1640 medium using a homogenizer. Bone marrow cells from 4- to 6-wk-old females were flushed from the tibia and femur with medium. Macrophages were obtained after washing the peritoneal cavity of diabetic donors with 5–10 ml of RPMI medium and depletion in β-cells by IgG panning and in T-cells by anti-Thy-I + C′ treatment. After one wash, cell viability was determined by Trypan blue exclusion during counting in a hemocytometer. After removal of β-cells and adherent cells by panning on goat anti-mouse Ig antibody–coated (Nordic Immunology, Tébu, Paris, France) petri dishes (100 μg purified Ig/dish), $CD4^+$ and $CD8^+$ spleen or LN T-cells were isolated by direct panning as well. Cloned GK 1.5 (anti-CD4) and cloned 53–6.7 (anti-CD8) were used as antibody producer cells. Each T-cell subset was found to be contaminated 2–3% with cells of the eliminated subpopulation.

Adoptive transfer. In each experimental group, 6–10 irradiated recipient mice were injected intravenously with donor cells, either unmodified or after cell depletions with various monoclonal antibodies as mentioned above. Spleen cells (5 or 10 × 10^6) and LN cells (5–20 × 10^6) were injected. Macrophages and total bone marrow cells were coinjected at a dose of 10^6 cells/mouse. Adoptive transfer of diabetes by using mixtures of purified $CD4^+$ and $CD8^+$ T-cells was performed in thymectomized animals. The $CD4^+$:$CD8^+$ ratio of the mixture was 2. Recipients were tested twice a week for glycosuria. After a positive urine test, blood glucose levels were determined with test strips and by a quantitative colorimetric assay (Reflolux, Boehringer, Mannheim). Animals showing glycemia >300 mg/dl were classified as overtly diabetic. Animals who died without developing diabetes were excluded from analysis.

Histopathology. At different times after cell transfer, 5–6 pancreases per group were fixed in Bouin's solution and processed for paraffin embedding. Four sections (5 μm) taken at 100-μm intervals were stained according to the hematein-eosin-safranin method. The proportion of infiltrated islets was based on the observation of 20–120 islets per specimen.

Statistical analysis. Pooled data were computed as means ± SE and were compared using Student's *t* test or χ^2 test.

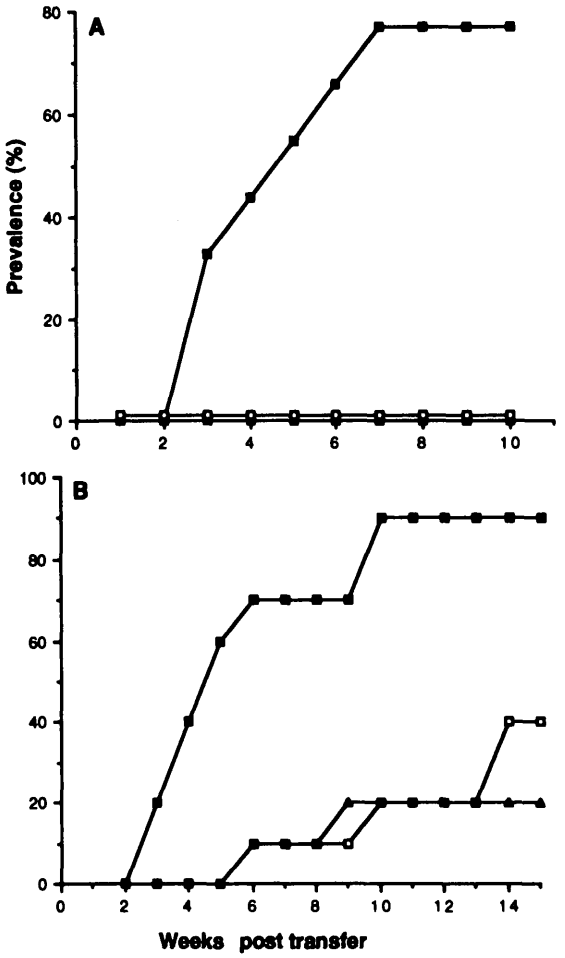

FIG. 1. Transfer of diabetes with LN cells from diabetic female mice. *A*: Unseparated cells from spleen (■, 5 × 10^6 cells, control) or from mesenteric and peripheral lymph nodes (□, 20 × 10^6, $P < 0.01$ vs. control) (△, 5 × 10^6 cells, $P < 0.05$ vs. control) were injected intravenously into irradiated male mice. *B*: 10 × 10^6 cells from spleen (■, control), pancreatic lymph nodes (□, $P < 0.01$ vs. control), or mesenteric and peripheral lymph nodes (△, $P < 0.01$ vs. control) were transferred to recipients. Statistical comparisons were made at the end of the experiment by χ^2 test (with Yates correction when necessary).

RESULTS

LN cell potential to induce insulitis and transfer diabetes. Figures 1 and 2 show three of four independent experiments performed to test the ability of LN cells to transfer diabetes. Cells pooled from mesenteric and peripheral lymph nodes of diabetic donors did not transfer diabetes as efficiently as splenic cells. This was observed whether 5–10 or 20 × 10^6 mesenteric and peripheral LN cells were injected (Fig. 1*A* and *B*). In two experiments, similar results were obtained when pancreatic LN cells were injected (Figs. 1*B* and 2). Onset of diabetes after transfer of mesenteric and peripheral LN cells was delayed for 2, 3, and 6 wk in three experiments, and no mice became diabetic in the fourth experiment. Accumulated data of the 10th wk posttransfer (Table 1), that is, when the prevalence of diabetes was maximum in all control groups injected with spleen cells (89%), show that only 20% of the mice injected with mesenteric and peripheral lymph nodes and 5% of the animals transferred with pancreatic lymph nodes were diabetic. At the

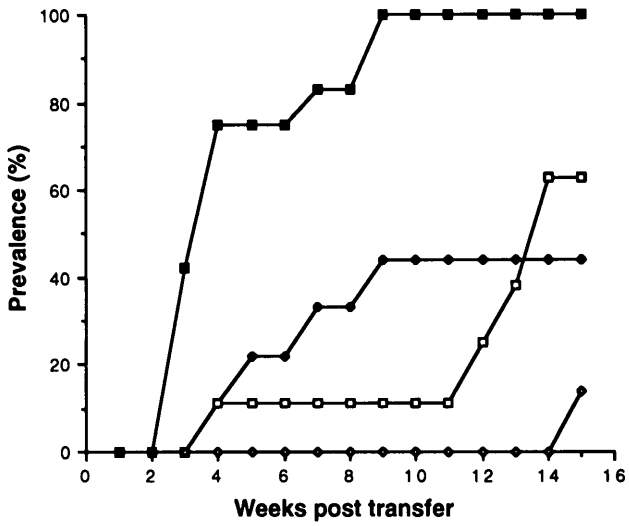

FIG. 2. Irradiated male mice were reconstituted with a mixture of spleen and mesenteric and peripheral LN cells (10^7 cells from each organ) (■, control), a mixture of 10^7 mesenteric and peripheral LN cells and 10^6 peritoneal macrophages (□, $P > 0.05$ vs. control), 10^7 mesenteric and peripheral LN cells (◆, $P < 0.02$ vs. control), and 8×10^6 pancreatic LN cells (◇, $P < 0.001$ vs. control). Statistical analysis was done at week 15.

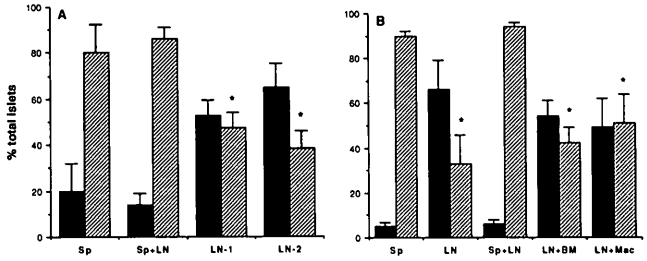

FIG. 3. Induction of insulitis after transfer of cells from diabetic female mice. The percentages of intact (■) vs. infiltrated (▨) islets are shown. *A*: Pancreases were analyzed 10 wk after the transfer of 5×10^6 spleen cells (*Sp*), 20×10^6 LN cells (*LN-1*), 5×10^6 LN cells (*LN-2*), or a mixture of spleen and LN cells (5×10^6 and 20×10^6 cells, respectively) (*Sp + LN*). *B*: Pancreases were analyzed 4 wk posttransfer. Mice received 10^7 spleen cells, or 10^7 mesenteric and peripheral LN cells, or 10^7 mesenteric and peripheral LN cells mixed with either 10^7 spleen cells, 10^7 bone marrow cells (*LN + BM*), or 10^6 peritoneal macrophages (*LN + Mac*). *$P < 0.03$ vs. spleen.

15th wk posttransfer, diabetes had developed in 32 and 28% of the mice injected with mesenteric and peripheral lymph nodes and pancreatic lymph nodes, respectively, whereas transfer was successful in 94% of the control mice (Table 1).

The degree of infiltration of the islets was determined 4 and 10 wk after transfer. The incidence of diabetes correlates well with the severity of insulitis. Whereas 80–90% of islets from recipients injected with spleen cells showed lymphocytic infiltration, only 30–45% of inflamed islets were observed in recipients of LN cells (Fig. 3). Based on the above results, we addressed three questions: Is the inefficiency of LN cells to transfer diabetes attributable to *1*) immunosuppression, *2*) the absence of or a difference in non–T-cell subsets compared with the spleen, or *3*) a difference in spleen and LN T-cell subset functions?

LN cells do not prevent the expression of overt diabetes. Mesenteric and peripheral LN cells (10^7 cells) together with 10^7 spleen cells, both from diabetic females, were injected into recipients. All mice became diabetic before the 10th wk posttransfer (Fig. 2), indicating that LN cells do not protect against diabetes development. Figure 3*B* also shows that LN cells, when mixed with spleen cells, do not prevent early infiltration of islets.

Macrophages and precursor cells do not promote transfer of disease by LN cells. Macrophages may quantitatively and qualitatively differ in the lymph nodes and spleen of diabetic NOD mice. In addition, the spleen contains hematopoietic stem cells and precursors that are not found in the lymph nodes. We therefore tested whether these nonlymphoid cell populations, when cotransferred with LN cells, could modify the effectiveness of LN cells in transfer experiments. Neither enriched peritoneal macrophages from diabetic mice (Fig. 2) nor total bone marrow cells from young, nondiabetic female mice (Fig. 4) significantly accelerated transfer of overt diabetes by LN cells for at least 10 wk. However, 15 wk posttransfer, the overall incidence of diabetes in the group that received macrophages and LN cells was increased (Fig. 2). Accumulated data from the two experiments are shown in Table 1. In addition, bone marrow cells as well as macrophages have no significant effect on the induction of insulitis by LN cells (Fig 3*B*). In separate experiments, none of the recipients of macrophages, bone marrow, or spleen cells from nondiabetic donors (4- to 6-wk old) developed diabetes, whereas 90–100% of the mice injected with spleen cells from diabetic females became diabetic.

TABLE 1
Prevalence of diabetes after transfer of different cell types

	Spleen cells	Mesenteric and peripheral LN cells	Pancreatic LN cells	LN plus macrophage cells	LN plus bone marrow cells
10 wk after transfer					
Prevalence of diabetes (%)	89	20	5	38	0
No. of diabetic mice	24 of 27	7 of 34	1 of 18	5 of 13	0 of 7
P value		<0.001	<0.001	<0.01	<0.001
15 wk after transfer					
Prevalence of diabetes (%)	94	32	28	69	14
No. of diabetic mice	17 of 18	8 of 25	5 of 18	9 of 13	1 of 7
P value		<0.001	<0.001	NS	<0.001

Transfer of spleen cells was compared with other experimental groups using χ^2 test with Yates correction when needed.

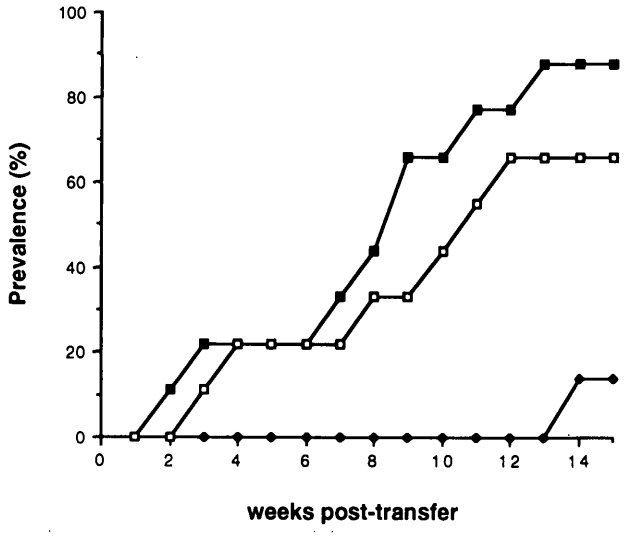

FIG. 4. Effect of bone marrow cells from young animals on the ability of LN cells from diabetic mice to transfer diabetes. Recipients were injected with a mixture of either 5 × 10^6 spleen cells from diabetic mice and 1 × 10^6 bone marrow cells from young females (■, control) or 5 × 10^6 spleen cells, 20 × 10^6 mesenteric and peripheral LN cells, and 1 × 10^6 bone marrow cells (□, $P > 0.05$ vs. control) or 20 × 10^6 mesenteric and peripheral LN cells and 1 × 10^6 bone marrow cells (◆, $P < 0.02$ vs. control).

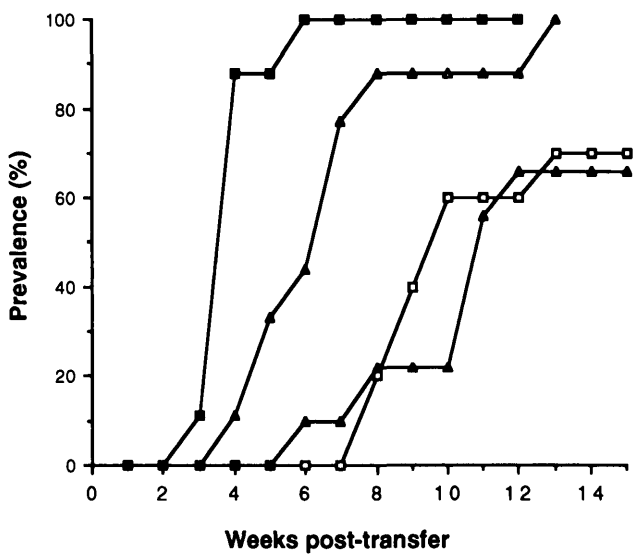

FIG. 5. Transfer of diabetes in recipients injected intravenously with 5 × 10^6 spleen T-cells (■), 5 × 10^6 mesenteric and peripheral LN T-cells (□), a mixture of 3 × 10^6 spleen $CD4^+$ cells and 1.5 × 10^6 LN $CD8^+$ cells (△), or a mixture of 1.5 × 10^6 spleen $CD8^+$ cells and 3 × 10^6 LN $CD4^+$ cells (▲).

LN $CD4^+$ T-cell subset cannot replace its splenic counterpart. To determine whether LN $CD4^+$, $CD8^+$, or both T-cell subsets, were deficient with regard to their ability to transfer disease, spleen and LN T-cells were depleted of $CD4^+$ or $CD8^+$ cells before transfer. Purified LN $CD4^+$ and $CD8^+$ cells were tested for their ability to replace the equivalent subset of spleen cells.

A mixture of spleen $CD4^+$ and LN $CD8^+$ cells transferred overt diabetes as well as purified unseparated spleen T-cells ($P > 0.05$). On the contrary, LN $CD4^+$ cells in conjunction with splenic $CD8^+$ cells transferred the disease with the same efficiency as the bulk of LN T-cells ($P < 0.05$ vs. spleen cell–injected recipients for both groups from the 4th up to the 10th wk posttransfer) (Fig. 5). Taken together, these data suggest that $CD4^+$ T-cells in lymph nodes differ from their counterparts in the spleen.

The transfer of total LN T-cells induced diabetes in 70% of animals by week 15 ($P > 0.05$ vs. control) but its development was delayed by several weeks compared to spleen cell recipients, which were all dead by week 11. Interestingly, because this experiment was performed with thymectomized animals, it shows that late onset of diabetes in LN cell recipients was not mediated by newly generated host T-cells. Insulitis was determined 3 wk posttransfer (Fig. 6). Inflammation did not differ between recipients of a mixture of $CD4^+$ spleen cells and $CD8^+$ LN cells and control animals. Conversely, insulitis was significantly less severe in mice coinjected with $CD4^+$ LN cells and $CD8^+$ spleen cells than in control mice ($P < 0.01$).

DISCUSSION

The presence of diabetogenic cells in NOD mouse spleen is evidenced by adoptive transfer of diabetes by splenic T-cells from mice >15 wk of age (8) and from diabetic mice (8–11). Both $CD4^+$ and $CD8^+$ T-cells are required for successful transfer, but their respective functions are not yet conclusively defined. However, evidence exists that spleen $CD4^+$ cells may act via cytokine release as helper cells for the activation of $CD8^+$ T-cells that mediate islet β-cell destruction (15); they also are required for $CD8^+$ cell homing in the islets (11,20). Herein, we show that LN cells from diabetic mice do not transfer diabetes as efficiently as spleen cells. The onset of diabetes mediated by LN cells was delayed by 2–10 wk or more, and the prevalence of diabetes varied at the 15th wk posttransfer from 20 to 60% among experiments. This phenomenon appeared to be dose independent in

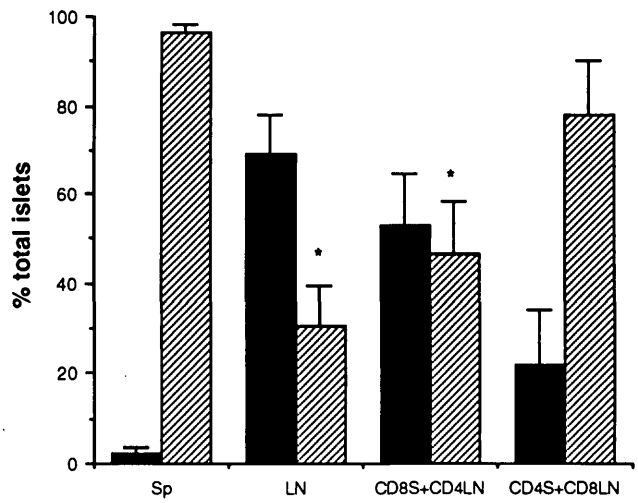

FIG. 6. Insulitis analysis in recipients of mixed subpopulations of spleen and LN cells. Pancreases were analyzed 4 wk after cell transfer. (*Sp*), Unseparated spleen T-cells; (*LN*), unseparated LN T-cells. The mean percentage of intact (■) and infiltrated (▨) are shown. *, $P < 0.01$ vs. spleen.

the range of 5–20 × 10^6 LN cells (that is, 4–15 × 10^6 T-cells); whereas as few as 2.5 × 10^6 spleen T-cells (from the same NOD colony) were shown to transfer diabetes efficiently (21). Late onset of diabetes was not mediated by newly generated host T-cells because the highest prevalence of disease was observed in thymectomized recipients of LN T-cells. An initial explanation for these data would be that lymph nodes contain a very low concentration of diabetogenic cells, compared with the spleen. Under normal conditions, memory T-cells tend to migrate preferentially through nonlymphoid tissues and inflammatory sites, whereas naive T-cells show a tendency to home preferentially to the lymph nodes (22). These distinct pathways of lymphocyte recirculation most likely result from the differential expression of adhesion molecules by the two populations (23). Nevertheless, memory T-cells enter lymph nodes via afferent lymph (24). The concentration of T-cells expressing a high density of CD44 molecules, which comprise memory T-cells, is only slightly decreased in the lymph nodes compared with the spleen (25). We observed similar results in NOD mice (data not shown). Thus, our results suggest that in NOD mice, functional β-cell–specific T-cells, unlike other memory cells, do not migrate into the lymph nodes as efficiently as into the spleen.

Interestingly, cells of the lymph nodes draining the pancreas were as inefficient as other LN cells in the transfer of diabetes. This observation may indicate either that only rare diabetogenic cells leave the pancreas and thus do not recirculate after having reached the target organ, or that recirculating pancreas-associated β-cell–specific T-cells are functionally modified. Thus, splenic diabetogenic cells may represent a cell population that has been chronically exposed to endogenous β-cell antigens, but which has not yet homed to the pancreas.

Another possibility is that nonlymphoid cells present in the transferred spleen cell populations, but absent or modified in the lymph nodes, are required for the development of disease in recipients. Macrophages are less numerous in the lymph nodes than in the spleen, and they may be of importance in the facilitation of β-cell destruction by immune cells (6,17,18). However, macrophages from diabetic mice did not accelerate the transfer of diabetes by LN cells, at least for 9–11 wk. In two separate experiments, the addition of macrophages resulted in an increase of diabetes prevalence after the 10th wk posttransfer. The possible role of macrophages in this late phenomenon remains to be clarified.

Similarly, hematopoietic stem cells and progenitors, absent from lymph nodes but present in the spleen and provided by bone marrow from young animals, did not help to improve the efficacy of lymph node diabetes mediators. The existence of suppressor T-cells that modulate the diabetic disease process has been shown in NOD mice (12,13,16) as well as in BB rats (26). Such immunosuppression was demonstrated in particular by using adoptive transfer experiments. When LN cells were transferred together with spleen cells, the kinetics of the development of diabetes in this group was not significantly different from that observed in the group reconstituted with spleen cells alone. Thus, the presence of suppressive cells specific for β-cell killers within the lymph nodes of diabetic mice seems unlikely.

Next, the question arises whether only one or both of the LN T-cell subsets were responsible for the low efficiency in the transfer of diabetes. Development of insulitis and diabetes in recipients of T-cells composed of LN $CD4^+$ and spleen $CD8^+$ cells or spleen $CD4^+$ and LN $CD8^+$ cells showed that $CD4^+$ and $CD8^+$ cells from the two organs are not interchangeable. These results suggest that although pathological LN and spleen $CD8^+$ T-cells are functionally indistinguishable, the $CD4^+$ T-cell subset seems to be altered in the lymph nodes. Further investigations are required to understand the functional difference between LN and spleen $CD4^+$ T-cells. The presence of diabetogenic cells in other lymphohemopoietic tissues in NOD mice is now under study.

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REFERENCES

1. Makino S, Kunimoto K, Muraoka Y, Mizushima Y, Katagiri K, Tochino Y: Breeding of a nonobese diabetic strain of mice. *Exp Anim* 29:1–13, 1980
2. Fujita T, Yui R, Kusumoto Y, Serizawa Y, Makino S, Tochino Y: Lymphocytic insulitis in a nonobese diabetic (NOD) strain of mice: an immunohistochemical and electron microscope investigation. *Biomed Res* 3:429–43, 1982
3. Ogawa M, Maruyama T, Hasegawa T, Kanaya T, Kobayashi F, Tochino Y, Uda H: The inhibitory effect of neonatal thymectomy on the incidence of insulitis in nonobese diabetic (NOD) mice. *Biomed Res* 6:103–105, 1985
4. Koike T, Itoh Y, Ishii T, Ito I, Takabayashi K, Maruyama N, Tomioka H, Yoshida S: Preventive effect of monoclonal anti-L3T4 antibody on development of diabetes in NOD mice. *Diabetes* 36:539–41, 1987
5. Sempe P, Bedossa P, Richard MF, Villa MC, Bach JF, Boitard C: Anti-α/β T-cell receptor monoclonal antibody provides an efficient therapy for autoimmune diabetes in nonobese diabetic (NOD) mice. *Eur J Immunol* 21:1163–69, 1991
6. Charlton B, Bacelj A, Mandel TE: Administration of silica particles or anti-Lyt 2 antibody prevents β-cell destruction in NOD mice given cyclosphosphamide. *Diabetes* 37:930–35, 1988
7. Mori Y, Suko M, Odudaira H: Preventive effects of cyclosporin on diabetes in NOD mice. *Diabetologia* 29:244–47, 1986
8. Wicker LS, Miller BJ, Mullen Y: Transfer of autoimmune diabetes mellitus with splenocytes from nonobese diabetic (NOD) mice. *Diabetes* 35:855–60, 1986
9. Bendelac A, Carnaud C, Boitard C, Bach JF: Syngeneic transfer of autoimmune diabetes from diabetic NOD mice to healthy neonates: requirement for both $L3T4^+$ and $Lyt\text{-}2^+$ T-cells. *J Exp Med* 166:823–32, 1987
10. Miller BJ, Appel MC, O'Neil JJ, Wicker LS: Both the $Lyt\text{-}2^+$ and $L3T4^+$ T-cell subsets are required for the transfer of diabetes in nonobese diabetic mice. *J Immunol* 140:52–58, 1988
11. Yagi H, Matsumoto M, Kunimoto K, Kawaguchi J, Makino S, Harada M: Analysis of the roles of $CD4^+$ and $CD8^+$ T-cells in autoimmune diabetes of NOD mice using transfer to NOD athymic nude mice. *Eur J Immunol* 22:2387–93, 1992
12. Boitard C, Yasunami R, Dardenne M, Bach JF: T-cell mediated inhibition of the transfer of autoimmune diabetes in NOD mice. *J Exp Med* 169:1669–80, 1989
13. Hutchings PR, Cooke A: The transfer of autoimmune diabetes in NOD mice can be inhibited or accelerated by distinct cell populations present in normal splenocytes taken from young males. *J Autoimmun* 3:175–85, 1990
14. Bradley BJ, Haskins K, La Rosa FG, Lafferty KJ: $CD8^+$ T-cells are not required for islet destruction induced by a $CD4^+$ islet-specific T-cell clone. *Diabetes* 41:1603–608, 1992
15. Nagata M, Yoon JW: Studies on autoimmunity for T-cell–mediated β-cell destruction: Distinct difference in β-cell destruction between $CD4^+$ and $CD8^+$ T-cell clones derived from lymphocytes infiltrating

the islets of NOD mice. *Diabetes* 41:998-1008, 1992
16. Pankewycz OG, Guan JX, Benedict JF: A protective NOD islet-infiltrating $CD8^+$ T-cell clone, I.S. 2.15, has in vitro suppressive properties. *Eur J Immunol* 22:2017–23, 1992
17. Lee KU, Amano K, Yoon JW: Evidence for initial involvement of macrophage in development of insulitis in NOD mice. *Diabetes* 37:989–91, 1988
18. Hutchings P, Rosen H, O'Reilly L, Simpson E, Gordon S, Cooke A: Transfer of diabetes in mice prevented by blockade of adhesion-promoting receptor on macrophages. *Nature* 348:639–42, 1990
19. Christianson SW, Shultz LD, Leiter EH: Adoptive transfer of diabetes into immunodeficient NOD-scid/scid micez: relative contributions of $CD4^+$ and $CD8^+$ T-cells from diabetic versus prediabetic NOD/NON-Thy-1^a donors. *Diabetes* 42:44–55, 1993
20. Bedossa P, Bendelac A, Bach JF, Carnaud C: 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* 19:1947–51, 1989
21. Yasunami R, Debray-Sachs M, Bach JF: Ontogeny of regulatory and effector T-cells in autoimmune NOD mice. In *Frontiers in Diabetes Research: Lessons From Animal Diabetes.* Vol. 3. Shafrir E, Ed. Smith-Gordorn, 1990, p. 88–93
22. Mackay CR, Marston WL, Dudler L: Naive and memory T-cells show distinct pathways of lymphocyte recirculation. *J Exp Med* 171:801–17, 1990
23. Lee WT, Vitetta ES: The differential expression of homing and adhesion molecules on virgin and memory T-cells in the mouse. *Cell Immunol* 132:215–22, 1991
24. Mackay CR, Kimpton WG, Brandon MR, Cahill RNP: Lymphocyte subsets show marked differences in their distribution between blood and the afferent and efferent lymph of peripheral lymph nodes. *J Exp Med* 167:1755–65, 1988
25. Budd RC, Cerottini JC, Horvath C, Bron C, Pedrazzini T, Howe RC, MacDonald HR: Distinction of virgin and memory T lymphocytes: stable acquisition of the Pgp-1 glycoprotein concomitant with antigenic stimulation. *J Immunol* 138:3120–29, 1987
26. Rossini A, Faustman A, Woda D, Like BA, Szymanski AA, Mordes JP: Lymphocyte transfusions prevent diabetes in the Bio-Breeding/Worcester rat. *J Clin Invest* 74:39–46, 1984