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Abnormal T cell selection on nod thymic epithelium is sufficient to induce autoimmune manifestations in C57BL/6 athymic nude mice

VÉRONIQUE THOMAS-VASLIN*, DIANE DAMOTTE†, MONIQUE COLTEY*, NICOLE M. LE DOUARIN*, ANTONIO COUTINHO‡, AND JOSSELYNE SALAÜN*§

*Institut d'Embryologie Cellulaire et Moléculaire, Centre National de la Recherche Scientifique, Collège de France, Nogent sur Marne, France; †Institut National de la Santé et de la Recherche Médicale, Unité 25, Hôpital Necker, Paris, France; and ‡Unité d'Immunobiologie, Centre National de la Recherche Scientifique, Unité de Recherche Associée 1961, Institut Pasteur, Paris, France

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ABSTRACT **To investigate the role of primary T cell repertoire selection in the immunopathogenesis of autoimmune diseases, pure thymic epithelium (TE) from nonobese diabetic (NOD) embryos was grafted into non autoimmune prone newborn C57BL/6 athymic mice. The results show that NOD TE selects host T cell repertoires that establish autoimmunity in otherwise nondiabetic animals. Thus, such chimeras regularly show CD4 and CD8 T cell-mediated insulitis and sialitis, in contrast with syngeneic or allogeneic chimeras produced with TE from nonautoimmune strains. This is the first demonstration that autoimmunity to pancreatic β cells and salivary glands can be established by the sole alteration of the thymic environment involved in T cell selection, regardless of the nature and presentation of both major histocompatibility complex and tissue-specific antigens on the target organ. These data indicate that T cell repertoire selection by the NOD thymic epithelium is sufficient to induce specific autoimmune characteristics in the context of an otherwise normal host.**

The nonobese diabetic (NOD) mouse provides an experimental model of human insulin-dependent diabetes mellitus (IDDM). These mice spontaneously develop a T cell-mediated autoimmune disease, characterized by a predominant inflammation of pancreatic islets followed by destruction of β cells, and diabetes, but affecting also other organs such as salivary glands (1–3) and thyroid (4, 5). The disease is primarily mediated by CD4 and CD8 T cells (6–14). Multiple genetic loci control NOD mice susceptibility to insulitis and autoimmune inflammation in other tissues, but full development of diabetes requires genes linked to major histocompatibility complex (MHC), namely the expression of the unique I-A^{NOD} (refs. 1, 15, 16, reviewed in ref. 17).

The immunopathogenic processes underlying IDDM are not fully elucidated. Attention has been given to the antigenic (MHC/peptide) composition of the target tissue, but the disease may as well result from a primary dysfunction of the immune system. In this case, failure of the mechanisms establishing self tolerance in the NOD mouse could result from altered repertoire selection either in the thymus, at the periphery, or both. Primary T cell repertoire selection occurs in the thymus, mediated by both epithelial and hemopoietic cells of the thymic stroma (for review see refs. 18–21). Several groups have suggested the role of thymic epithelium (22, 23) or hemopoietic cells (24–28) in the development of IDDM, but a putative differential role of each stromal cell type has not been addressed. Since positive and negative thymic selections have been preferentially associated with epithelial and hemopoietic antigen-presenting cells, respectively, the identification of the immunopathogenic role of either cell type could indicate the kinds of tolerance mechanisms that are defective in NOD mice.

We have now addressed some of those questions: (*i*) Is the nature and presentation of antigens in NOD pancreas critical to the development of autoimmunity, or can a similar condition be induced in normal mice by altering T cell repertoires? (*ii*) Does autoimmune pathology in NOD mice result from defects of thymic, or peripheral repertoire selection? (*iii*) If there is a thymic defect in NOD animals, which type of stromal cell (epithelial or hemopoietic) is responsible for the failure in establishing self-tolerance?

The following experimental approach was used. The third pharyngeal pouches from day 10 (24–28 somites) mouse embryos, just before colonization by hemopoietic precursor cells, constitute the rudiment of pure TE (29, 30). If such TE rudiments are transplanted to syngeneic or allogeneic athymic newborn hosts, they will be colonized by recipient's hemopoietic cells and the resulting chimeric thymus restores the peripheral T cell compartment of the nude host (31). As previously reported by us and others (refs. 31–34, reviewed in refs. 35–37), TE rudiments invariably establish transplantation tolerance to grafts of various tissues from the respective donor. This experimental protocol was adapted to address questions of IDDM pathogenesis, by restoring normal C57BL/6 (B6) nude mice with TE from autoimmune prone NOD embryos, and following autoimmune disease development in the chimeras. It should be underlined that we are dealing here with chimeric animals that are entirely B6, except for TE cells that remain localized in the site of grafting and cannot, therefore, participate in peripheral presentation of antigens.

MATERIALS AND METHODS

Mice. BALB/c (H-2^d), C57BL/6 (B6) (H-2^b), B6 athymic nu/nu (nude), and NOD (H-2^{g7}) mice, originally obtained from Bomholtgard, (Denmark), were bred in our animal facilities.

Chimeras. B6 nu/nu mice were grafted within the first week of life with either 16–20 pharyngeal pouches removed from embryonic day 10 (E10) embryos (24–28 somites), or 3–5 lobes of colonized thymus from E17 fetuses, under the skin of the neck as previously described (31). After 2–3 months, restoration of the T cell compartment was assessed by cytofluoro-

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Abbreviations: E*n*, embryonic day *n*; NOD: nonobese diabetic; IDDM, insulin-dependent diabetes melitus; B6(NOD E*n*): C57BL/6 athymic nude mouse restored by the graft of NOD E*n* thymic rudiments; MHC, major histocompatibility complex; TE, thymic epithelium.

§To whom reprint requests should be addressed.

metric quantitation of $CD4^+$ and $CD8^+$ T cells in peripheral blood lymphocytes. Only mice with at least 20% T cells in the lymphocyte population (defined by light scatter parameters) were considered restored and used in the present experiments. T cell restoration was routinely confirmed by cytometric analyses of spleen cells, at the time of sacrifice. On the 20 chimeras analyzed, six were males.

Assessment of Autoimmune Disease. Glycemia was scored by strip bands (BM-test glycemie, Boehringer Mannheim) in venous whole blood obtained by punction of the retroorbital sinus of mice that had fasted for ≈5 hr. After sacrifice, half of the organs were fixed in Bouin and processed for conventional histology (5 μm paraffin sections, hematoxylin/eosin/safran coloration), and estimation of the severity of cellular infiltrations. The other half of the organs was frozen in isopentane cooled in liquid nitrogen. Cryostat sections (8 μm) were processed for immunohistochemistry staining with rat anti-mouse CD4 (RM4-5, PharMingen) or CD8 (53-6-7, PharMingen) biotinylated antibodies. After washing, this step was followed by addition of monoclonal mouse anti-rat κ+λ-biotin-labeled antibody (Sigma) and after further washing the complexed avidin-biotin-peroxidase (Vectastain ELITE AB complex, Vector Laboratories) was added. Finally, the peroxidase activity was revealed by addition of the substrate 3-amino-9-ethylcarbazole (Sigma). Four to eight sections levels of pancreas and salivary glands, spaced of at least 200 μm, were inspected. In pancreas, 30–100 islets were examined, periinsulitis and insulitis quantified, and the severity of insulitis scored. The severity of sialitis was quantified in grades: 0, no infiltration; 1, sporadic infiltrations, one to two small foci per gland; 2, moderate infiltration, several small foci of infiltration through the gland; 3, severe sialitis, several large foci on the same section.

RESULTS

Experimental Protocol. The principle of the present approach was to find out whether the restoration at birth of athymic B6 nude mice with a pure TE from autoimmune prone NOD donors was predisposing the restored recipients to autoimmune disorders. Thymic rudiments were removed from embryos (E10) before hemopoietic stem cell colonization, which occurs from E11 (29), ensuring that no lymphocyte or professional antigen-presenting cell in the chimeras was derived from the TE grafts. This protocol allows for the differentiation and selection of recipient T cell precursors on NOD TE in the presence of host hemopoietic antigen-presenting cells. It assesses the tolerant vs. autoimmune behavior of the T cells thus selected, on the recipient (normal) organs. Additionally, the eventual contribution of NOD HC was investigated by grafting colonized thymus removed from E17 NOD fetuses.

B6 H-2^b nude mice were immunologically restored by allogeneic embryonic thymus grafts from E10 or E17 NOD embryos [denoted B6(NOD E10) and B6(NOD E17) respectively], or from BALB/c E10 embryos [denoted B6(BALB E10)]. Syngeneic TE grafts were also performed in a group of control animals [denoted B6(B6 E10)]. Such TE chimeras were previously shown (*i*) to develop chimeric thymus with normal histological structure, (*ii*) to produce functional T cells that repopulate peripheral lymphoid tissues and respond *in vitro* to mitogens and allogeneic stimulators, (*iii*) to reject third-party tissue grafts, and (*iv*) to be fully tolerant to skin and heart grafts of the TE haplotype (31, 33, 38). TE-induced tolerance is life long, cannot be "broken" by repeated immunizations with antigen-presenting cell from the tolerized donor, does not involve complete deletion of specific alloreactive T cells, and is mediated by regulatory T cells selected on the TE (33, 34, 39–43).

In the present experiments, the representation of mature T cells among the lymphocyte population in spleen at sacrifice (i.e., at 10–22 months) among the 20 nude mice analyzed was 8.7 ± 3.8% for CD4 and 9.9 ± 4.5% for CD8 T cells, with no significant variation from group to group.

Inflammatory Lesions in Pancreas and Salivary Glands. Autoimmune disorders in chimeric animals were assessed by histological observation of the pancreas and the salivary glands. Pancreas is an endocrine and exocrine organ in which the endocrine tissue is the only one to be pathological in NOD mouse. In the salivary glands, there is only exocrine tissue that may also be pathological in NOD mouse. In the pancreas of B6(NOD E10), infiltrations corresponding to periinsulitis and insulitis were scored (Fig. 1). The inflammatory lesions were focal, concerning only some islets. In B6(NOD E10) chimeras, we found insulitis and periinsulitis in 19–52% of the islets. Conversely, in control, age-matched syngeneic B6(B6 E10) and allogeneic B6(BALB E10) chimeras, we found only sparse lymphoid cells with a mean of 3.3% of islets presenting insulitis in spite of individual variation from mouse to mouse. This is also observed in aged normal B6 mice (44). The smaller group of B6(NOD E17) chimeras studied was heterogeneous, with two out of four animals showing significant pancreatic inflammatory lesions while the two others did not.

The data in Fig. 1 were analyzed by a nonparametric Mann–Whitney *U* test. This analysis demonstrates the significant augmentation of insulitis incidence (*P* corrected for ex-aequo = 0.001) and the significant augmentation in the percentage of inflamed islets (*P* corrected for ex-aequo = 0.003), in B6(NOD E10) chimeras ($n = 7$), as compared with control chimeras ($n = 9$).

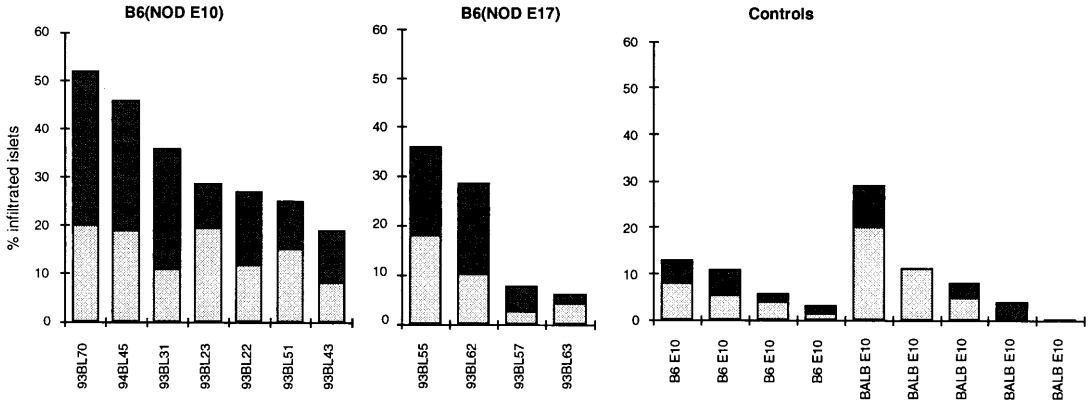

FIG. 1. Infiltration in the pancreas of B6 nude mice restored by the graft of NOD E10, NOD E17, or B6 E10 and BALB/c E10 thymic rudiments as controls. The percentage of periinsulitis (■) and insulitis (▨) is indicated for individual chimeras, as determined by observation of classical paraffin histological sections.

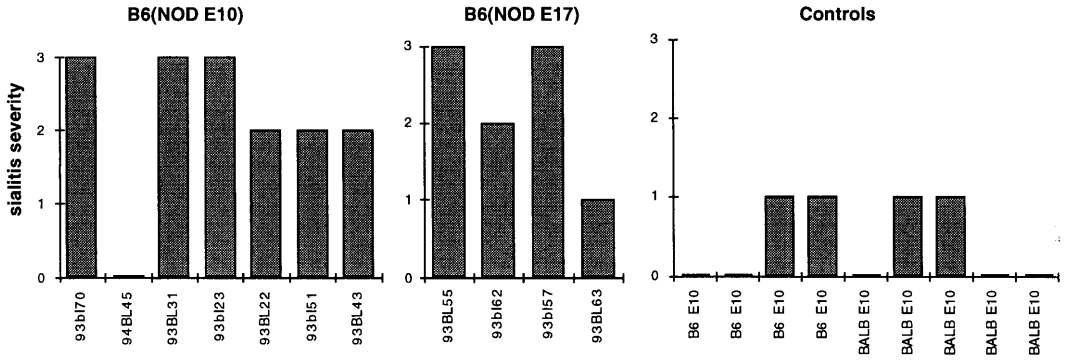

FIG. 2. Infiltration in salivary glands of B6 nude mice restored by the graft of NOD E10, NOD E17, or B6 E10 and BALB/c E10 thymic rudiments as controls. The severity of sialitis is indicated as described in *Materials and Methods*.

All chimeras were also studied for inflammatory infiltrates in the salivary glands, known to accompany the pathological manifestations in NOD mice. As can be seen in Fig. 2, all of the nude B6 mice reconstituted with NOD thymic rudiments, showed moderate to severe mononuclear cell infiltrations. In contrast, we found no sialitis or only discrete infiltrates in control chimeras, as it is often found in normal old mice (44).

Figs. 1 and 2 also show that in most chimeras the degree of insulitis and sialitis correlated well in individual mice. The severity of insulitis and sialitis was not correlated with the sex of the chimeras.

Characterization of CD4 and CD8 Infiltration in Pancreas and Salivary Glands. Both CD4 and CD8 T cells were reported to play an important role in the inflammation and destruction of pancreatic islets (6–13). Moreover, the inflammatory infiltrates of the spontaneous disease in NOD mice include both types of cells. We have analyzed, therefore, the phenotype of cells infiltrating the pancreas and the salivary glands of the TE chimeras.

Immunohistological staining with anti-CD4 and anti-CD8 antibodies reveals the presence of both CD4 and CD8 infiltrating T lymphocytes, surrounding and invading the islets (Fig. 3), and infiltrating the salivary glands of B6 (NOD E10) chimeras (Fig. 4). In both pancreatic and salivary infiltrates of these chimeras, CD4 T cells were usually more abundant than CD8 T cells.

Resistance of Chimeras to Overt Diabetes. The glycemia of all chimeras was monitored regularly, from 2 months to sacrifice. In some mice, we noted increases in fasting venous whole blood glucose levels, sometimes reaching up to 1.6–2 g/l, 2–6 months after thymic grafting. In all cases, however, the glycemia always returned to normal values after 1 week.

Treatment with cyclophosphamide is known to accelerate the onset of diabetes in prediabetic NOD mice (45). We have investigated, therefore, whether such treatments could precipitate overt diabetes in similar chimeras. Two (days 1, 14) or four (days 1, 14, 28, 43) consecutive injections of 200 mg/kg cyclophosphamide administered intraperitoneally did not result, however, in the establishment of stable hyperglycemia in any of the 14 chimeras treated. However, a low grade of insulitis was present in 50% of that group (data not shown).

DISCUSSION

The present experiments investigated the specific role of TE in the pathogenesis of cell-mediated autoimmune disorders. More precisely, we asked whether B6 T cells, selected in a chimeric thymus which contains a TE of NOD origin would mediate insulitis and sialitis in B6 mice. The results show that all B6(NOD E10) chimeras developed insulitis and sialitis, with the single exception of a mouse that presented inflammation of the pancreas but not of the salivary glands. In contrast, control chimeras, constructed with an allogenic BALB/c or a syngeneic B6 TE were essentially free of inflammatory infiltrates. The severity of sialitis was an important parameter of autoimmunity in B6(NOD E10) chimeras, as it is reported that salivary gland inflammation is more severe and occurs earlier than insulitis in NOD.H-2^b congenic mice (1). Although multiple organ autoimmunity may occur spontaneously in aged mice of the B6 strain (44) and this may explain the sporadic infiltrates scored in control chimeras, the pathology observed in B6(NOD E10) animals is significantly more important.

These observations, therefore, demonstrate that NOD TE alone can select an antoimmune T cell repertoire. This is the first formal demonstration of the direct role of NOD TE in the induction of autoimmune manifestations, but the present results, by limiting to TE the entire participation of the autoimmune strain in the chimeras, also strongly suggest that other factors play roles in IDDM development. The fact that, in B6(NOD E10) chimeras, inflammatory infiltration by B6 T lymphocytes occur in pancreas and salivary glands that are entirely B6 suggests that the nature and presentation of antigens in peripheral tissues (parenchymal or professional presenting cells) is not critical to the initiation of autoimmunity. The crucial requirement for I-A^{NOD} in diabetes development is likely, therefore, to concern deficient thymic selection of T cell repertoires, rather than presentation of antigenic peptides in the pancreas and salivary glands. Leijon *et al.* (46) had previously shown in tetraparental NOD/B6 chimeras grafted with NOD and B6 islets that animals that destroyed NOD islets, also destroyed B6 islets by a secondary unrestricted inflammatory process, as also reported by others (47, 48). These results also indicated that NOD islets are not intrinsically more "autoantigenic" than normal non NOD islets. One could however neither exclude that the initiation of tissue infiltration required NOD tissue-specific antigens nor that NOD presenting cells had migrated to the B6 graft. Our observations rule out these reservations.

The role of thymic selection in rodent diabetic models has previously been addressed by several groups. Most of these studies, however, used highly manipulated rat or mouse chimeras, produced by adult thymectomy, irradiation bone marrow reconstitution, and grafting with irradiated or deoxyguanosin treated thymus. Another problem with these experimental systems is that purity of TE and its functional competence cannot be ascertained. This may explain the contradictory results obtained, namely, protection or lack of it, either mediated by TE or by hemopoietic cells from diabetes-resistant animals (23, 24, 26, 28). Similarly, disease induction by NOD bone marrow cells transfered to irradiated F1 nonsusceptible recipients was reported (25, 28, 49). The transfer of F1 spleen cells to NOD newborn mice led to prevention of disease (27). The role of NOD TE in the positive selection of pancreatic β cell-directed autoreactive T cell repertoires was previously suggested by observations in allophenic NOD<-

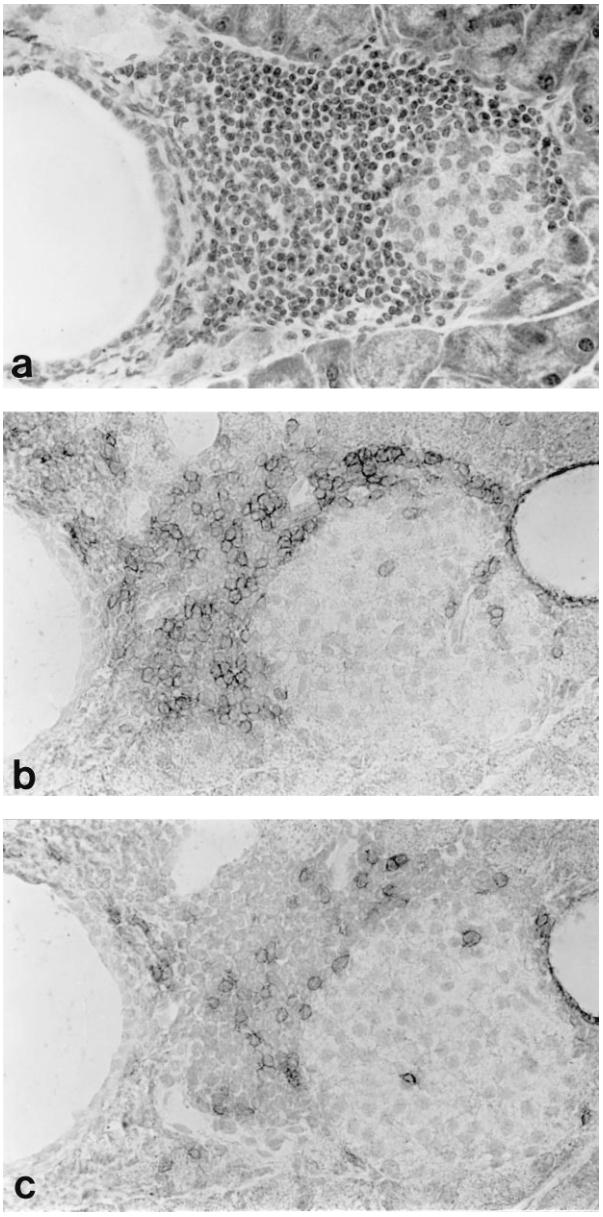

FIG. 3. Pancreatic histology of frozen pancreas sections of B6(NODE10) chimeras. (*a*) Hematoxylin, eosin and saffron coloration showing lymphocytes in an infiltrated islet. CD4 (*b*) and CD8 (*c*) lymphocytes labeled with specific monoclonal antibodies in the same islet. (×290.)

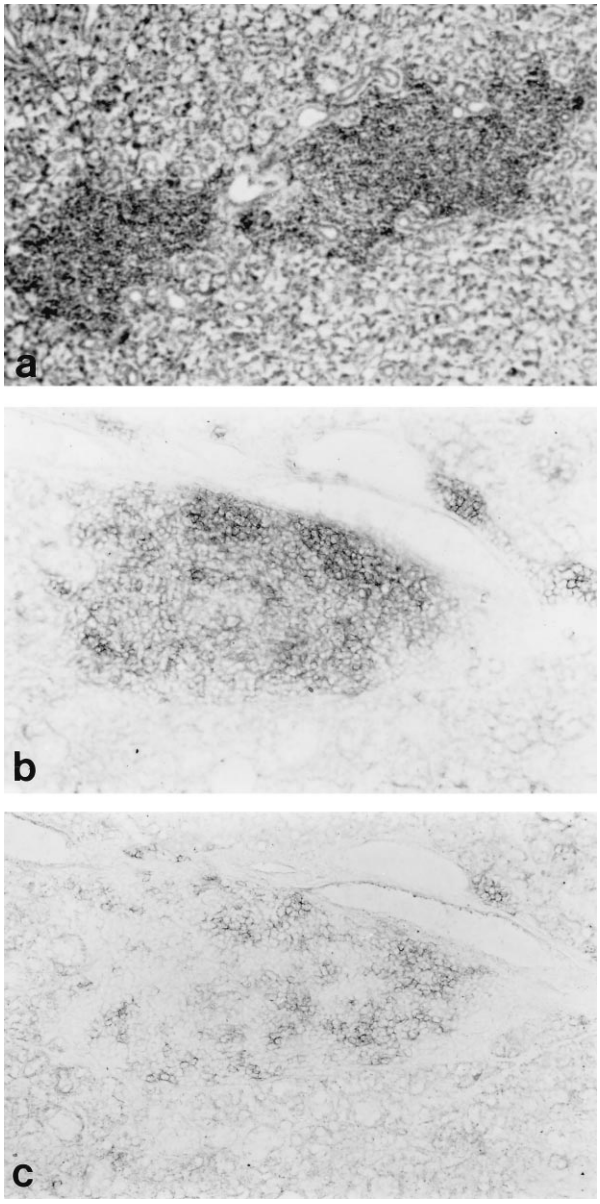

FIG. 4. Histology of frozen salivary gland sections of B6(NODE10) chimeras. (*a*) Hematoxylin eosin coloration showing two large infiltrates. (×100.) Immunohistological staining of CD4 (*b*) and CD8 (*c*) lymphocytes labeled with specific monoclonal antibodies in an infiltrate. (×140.)

>B6 mice, where the representation of NOD cells in the cortical thymus correlated with the development of insulitis (22).

Since altered thymic selection on NOD TE is sufficient for initiation of autoimmune pathology, putative defects in peripheral tolerance mechanisms seem less attractive as an explanation for disease development. Furthermore, the present demonstration of the pathogenic role of pure NOD TE (while NOD E17 thymus graft does not significantly modify the pathology observed) shows that hemopoietic antigen-presenting cells in NOD thymus are not likely to play a major role in selecting a IDDM-prone T cell repertoire. Given that TE has been preferentially associated with positive selection and that hemopoietic-presenting cells selectively mediate clonal deletions, the present findings suggest that NOD mice suffer from a primary anomaly in thymic positive selection of T cells. This speculation is in line with our previous demonstration that TE induces tolerance in allogeneic recipients because it selects for regulatory CD4 T lymphocytes that mediate dominant tolerance (refs. 34, 39–41, reviewed in refs. 37, 50). Together with these and other observations on the dominant regulation of tissue-specific pathogenic T cells in normal individuals (51–54), the present results could be interpreted to suggest that the origin of spontaneous IDDM development in NOD mice is due to the failure of TE to select an appropriate repertoire of regulatory T cells (which control autoreactive T cells as shown by others, refs. 55 and 56). It remains to be established whether such putative difference in the ability of NOD TE in selecting regulatory T cells is qualitative, quantitative, or both. As previously demonstrated, young NOD mice do contain regulatory T cells that delay diabetes development when transfered in older mice (57), and their elimination has been invoked to explain induction of diabetes in young animals by cyclophosphamide treatments (45). Such regulatory T cells, however, are insufficient to prevent disease development in most NOD animals. The

critical role of I-A^{NOD} in diabetes development (1, 15, 16, 58) could also be interpreted in the light of the present hypothesis. Thus, class II transgenic NOD mice expressing non-NOD I-A molecules do not develop diabetes although they continue to express their endogenous I-A^{NOD} (55, 56). In other words, non-NOD IA class II molecules are dominant over I-A^{NOD} in inducing physiological self-tolerance. While these results have been interpreted by postulating competition between NOD and transgenic class II molecules for the presentation of "immunogenic" self-peptides, it could also be proposed that transgenic class II molecules positively select the set of regulatory CD4 T cells, which I-A^{NOD} fails to do.

The particular MHC haplotype H-2^{g7} presented on the NOD TE might be responsible for the selection of autoreactive T cells. Recent observations that I-A^{g7} and the pancreatic autoantigen GAD-65, present similarities in a decapeptide (59) and that pro-insulin and GAD-65 also present common protein sequences (60) suggest that the community between the three molecules on 10-amino acid length might result in selection of autoreactive T cells. Alternatively, I-A^{g7} might present a unique set of peptides, resulting in selection of autoreactive aggressive CD4 T cells rather than in regulatory CD4 T cells. Non-MHC restricted inflammation of the target cells (as previously discussed) or cross reactivity between allo-MHC molecules as reported by others (27) may then occur. Independent of I-A^{g7} expression on NOD-TE, the reduced expression of class I-molecules in NOD might affect the T cell repertoire (61).

The absence of overt diabetes in the B6(NOD TE) chimeras may have a variety of explanations. First of all, this result could actually be expected, since B6 does not express susceptible alleles at nearly all the many genetic loci that control susceptibility to diabetes (1). The initiation of the autoimmune process and tissue inflammation may well be determined by T cell repertoires, but many other processes (not necessarily immunological) may be required for progression to overt disease. Secondly, diabetes is only established when >80% of all islets are destroyed, and the autoaggressive T cell repertoires in B6(NOD TE) chimeras may not reach the required quantitative levels. Thus, the frequency of effector T cells might be reduced in the chimeras, due to partial MHC compatibility between the TE and the peripheral tissues. In these conditions, of all CD4 T cells educated on I-A^{NOD}, only those crossreacting with I-A^{b} can possibly function in the periphery. For CD8 T cells, only D^{b} is shared between the two haplotypes, while islets associated cytotoxic T lymphocytes are mainly restricted to K^{d} (12, 14, 62). Finally, we have noted a predominance of CD4 T cells in the B6(NOD E10) chimera infiltrates, and an imbalance between CD4 and CD8 effector cells may also be invoked to explain lack of progression to overt diabetes. Thus, CD4 cells are often thought to be involved in insulitis but not in diabetes (10), or as protecting from diabetes, although not from insulitis (57). Insulitis also persists after remission of overt diabetes induced by anti-CD3 antibody treatments of NOD mice (63).

Further experiments are required to investigate the mechanisms leading from a pathogenic T cell repertoire selected on NOD TE to the full destruction of β cells. The model described here may be of value in this endeavor.

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1. Wicker, L. S., Appel, M. C., Dotta, F., Pressey, A., Miller, B. J., DeLarato, N. H., Fisher, P. A., Boltz, R. C. & Peterson, L. B. (1992) *J. Exp. Med.* **176,** 67–77.
2. Miyagawa, J., Hanafusa, T., Miyazaki, A., Yamada, K., Fujino-Kurihara, H., Nakajima, H., Kono, N., Nonaka, K., Tochino, Y. & Tarui, S. (1986) *Virchows Arch. B Cell Pathol.* **51,** 215–225.
3. Goillot, E., Mutin, M. & Touraine, J. L. (1991) *Clin. Immunol. Immunopathol.* **59,** 462–473.
4. Bernard, N. F., Ertug, F. & Margolese, H. (1992) *Diabetes* **41,** 40–46.
5. Many, M. C., Maniratunga, S., Varis, I., Dardenne, M., Drexhage, H. M. & Denef, J. F. (1995) *J. Endocrinol.* **147,** 311–320.
6. Bendelac, A., Carnaud, C., Boitard C. & Bach, J. F. (1987) *J. Exp. Med.* **166,** 823–832.
7. Bedossa, P., Bendelac, A., Bach, J. F. & Carnaud, C. (1989) *Eur. J. Immunol.* **19,** 1947–1951.
8. Gill, R., Rosenberg, A. S., Lafferty, K. J. & Singer, A. (1989) *J. Immunol.* **143,** 2176–2178.
9. Thivolet, C., Bendelac, A., Bedossa, P., Bach, J.-F. & Carnaud, C. (1991) *J. Immunol.* **146,** 85–88.
10. Yagi, H., Matsumoto, M., Kunimoto, K., Makino, S. & Harada, M. (1992) *Eur. J. Immunol.* **22,** 2387–2393.
11. Katz, J., Benoist, C. & Mathis, D. (1993) *Eur. J. Immunol.* **23,** 3358–3360.
12. Nagata, M., Santamaria, P., Kawamura, T., Utsugi, T. & Yoon, J. W. (1994) *J. Immunol.* **152,** 2042–2050.
13. Katz, J., Benoist, C. & Mathis, D. (1995) *Science* **268,** 1185–1188.
14. Wong, S., Visintin, I., Wen, L., Flavell, R. & Janeway, C. A., Jr. (1996) *J. Exp. Med.* **183,** 67–76.
15. Wicker, L. S., Beverly, J. M., Coker, L. Z., McNally, S. E., Scott, S., Mullen, Y. & Appel, M. C. (1987) *J. Exp. Med.* **165,** 1639–1654.
16. Todd, J. A., Aitman, T. J., Cornal, R. J., Ghosh, S., Hall, J. R. S., Hearne, C. M., Knight, A. M., Love, J. M., McAleer, M. A., Prins, J.-B., Rodrigues, N., Lathrop, M. Pressey, A., Delarato, N. H., Peterson, L. B. & Wicker, L. S. (1991) *Nature* **351,** 542–547.
17. Serreze, D. V. & Leiter, E. H. (1994) *Curr. Opin. Immunol.* **6,** 900–906.
18. Elliott, J. I. (1993) *Immunol. Rev.* **135,** 215–225.
19. Hogquist, K. A., Jameson, S. C. & Bevan, M. J. (1994) *Curr. Opin. Immunol.* **6,** 273–278.
20. von Boehmer, H. (1994) *Cell* **76,** 219–228.
21. Rothenberg, E. V. (1994) *Curr. Opin. Immunol.* **6,** 257–265.
22. Forsgren, S., Dahl, U., Soderstron, A., Holmberg, D. & Matsunaga, T. (1991) *Proc. Natl. Acad. Sci. USA* **88,** 9335–9339.
23. Georgiou, H. M. & Mandel, T. E. (1992) *Transplant. Proc.* **24,** 203–204.
24. Ikehara, S., Ohtsuki, H., Good, R. A., Asamoto, H., Nakamura, T., Sekita, K., Muso, E., Tochino, Y., Ida, T., Kuzuya, H., Imura, H. & Hamashima, Y. (1985) *Proc. Natl. Acad. Sci. USA* **82,** 7743–7747.
25. Wicker, L. S., Miller, B., Chai, A., Terada, M. & Mullen, Y. (1988) *J. Exp. Med.* **167,** 1801–1810.
26. Georgiou, H. M. & Bellgrau, D. (1989) *J. Immunol.* **142,** 3400–3405.
27. Bendelac, A., Boitard, C., Bach, C. & Carnaud, C. (1989) *Eur. J. Immunol.* **19,** 611–616.
28. Serreze, D. V. & Leiter, E. H. (1991) *J. Immunol.* **147,** 1222–1229.
29. Fontaine-Pérus, J. C., Calman, F. M., Kaplan, C. & Le Douarin, N. M. (1981) *J. Immunol.* **126,** 2310–2316.
30. Jotereau, F., Heuze, F., Salomon-Vié, V. & Gascan, H. (1987) *J. Immunol.* **138,** 1026–1030.
31. Salaün, J., Bandeira, A., Khazaal, I., Calman, F., Coltey, M., Coutinho, A. & Le Douarin, N. M. (1990) *Science* **247,** 1471–1474.
32. Hoffmann, M. W., Allison, J. & Miller, J. F. (1992) *Proc. Natl. Acad. Sci. USA* **89,** 2526–2530.
33. Thomas-Vaslin, V., Salaün, J., Gajdos, B., Le Douarin, N., Coutinho, A. & Bandeira, A. (1995) *Eur. J. Immunol.* **25,** 438–445.
34. Modigliani, Y., Thomas-Vaslin, V., Bandeira, A., Coltey, M., Le Douarin, N. M., Coutinho, A. & Salaün, J. (1995) *Proc. Natl. Acad. Sci. USA* **92,** 7555–7559.
35. Le Douarin, N. M., Corbel, C., Martin, C., Coltey, M. & Salaün, J. (1989) *Cold Spring Harbor Symp. Quant. Biol.* **65**, 777–787.
36. Coutinho, A., Salaün, J., Corbel, C., Bandeira, A. & Le Douarin, N. (1993) *Immunol. Rev.* **133,** 225–240.

37. Le Douarin, N. M., Corbel, C., Bandeira, A., Thomas-Vaslin, V., Modigliani, Y., Coutinho, A. & Salaün, J. (1996) *Immunol. Rev.* **149,** 35–53.
38. Khazaal, I., Salaün, J., Coltey, M., Calman, F. & Le Douarin, N. (1989) *Cell Differ. Dev.* **26,** 211–220.
39. Modigliani, Y., Pereira, P., Thomas-Vaslin, V., Salaün, J., Burlen-Defranoux, O., Coutinho, A., Le Douarin, N. & Bandeira, A. (1995) *Eur. J. Immunol.* **25,** 2563–2571.
40. Modigliani, Y., Coutinho, A., Pereira, P., Le Douarin, N. M., Thomas-Vaslin, V., Salaün, J. & Bandeira, A. (1996) *Eur. J. Immunol.* **26,** 1807–1815.
41. Thomas-Vaslin, V., Coltey, M. & Salaün, J. (1996) *C.R. Acad. Sci. Ser. III* **319,** 401–404.
42. Salaün, J., Bandeira, A., Khazaal, I., Burlen-Defranoux, O., Thomas-Vaslin, V., Coltey, M., Le Douarin, N. M. & Coutinho, A. (1992) *Proc. Natl. Acad. Sci. USA* **89,** 10420–10424.
43. Bandeira, A., Coutinho, A., Burlen-Defranoux, O., Khazaal, I., Coltey, M., Jacquemart, F., Le Douarin, N. M. & Salaün, J. (1992) *Eur. J. Immunol.* **22,** 1397–1404.
44. Ahmed, S. A. (1994) *Immunol. Today* **15,** 389–390.
45. Yasunami, R. & Bach, J. F. (1988) *Eur. J. Immunol.* **18,** 481–484.
46. Leijon, K., Hillörn, V., Bergqvist, I. & Holmberg, D. (1995) *J. Autoimmun.* **8,** 347–356.
47. Serreze, D. V., Leither, E. H. & Schultz, L. D. (1990) *Diabetologia* **33,** 84–92.
48. Kolb, H., Kolb-Bachofen, V. & Roep, B. O. (1995) *Immunol. Today* **16,** 170–172.
49. Serreze, D. V., Leither, E. H., Worthen, S. M. & Shultz, L. D. (1988) *Diabetes* **37,** 252–255.
50. Modigliani, Y., Bandeira, A. & Coutinho, A. (1996) *Immunol. Rev.* **149,** 155–174.
51. Sakaguchi, S., Takahashi, T. & Nishizuka, Y. (1982) *J. Exp. Med.* **156,** 1577–1586.
52. Sakaguchi, S., Fukuma, K., Kuribayashi, K. & Masuda, T. (1985) *J. Exp. Med.* **161,** 72–87.
53. Powrie, F. & Mason, D. (1990) *J. Exp. Med.* **172,** 1701–1708.
54. Lo, D., Burkly, L. C., Flavell, R. A., Palmiter, R. D. & Brinster, R. L. (1989) *J. Exp. Med.* **170,** 87–104.
55. Singer, S. M., Tisch, R., Yang, X. D. & McDevitt, H. O. (1993) *Proc. Natl. Acad. Sci. USA* **90,** 9566–9570.
56. Slattery, R. M., Miller, J. F. A. P., Heath, W. R. & Charlton, B. (1993) *Proc. Natl. Acad. Sci. USA* **90,** 10808–10810.
57. Boitard, C., Yasunami, R., Dardenne, M. & Bach, J. F. (1989) *J. Exp. Med.* **169,** 1669–1680.
58. Hattori, M., Buse, J. B., Jackson, R. A., Glimcher, L., Dorf, M. E., Minami, M., Makino, S., Moriwaki, K., Kuzuya, H., Imura, H., Strauss, W. M., Seidman, J. G. & Eisenbarth, G. S. (1986) *Science* **231,** 733–735.
59. Baum, H. & Cunningham, P. (1994) *Immunol. Today* **15,** 388–389.
60. Rudy, G., Brusic, V., Harrison, L. C. & Lew, A. M. (1995) *Immunol. Today* **16,** 406–407.
61. Li, F., Guo, J., Fu, Y., Yan, G. & Faustman, D. (1994) *Proc. Natl. Acad. Sci. USA* **91,** 11128–11132.
62. Nagata, M., Yokono, K., Hayakawa, M., Kawase, Y., Hatamori, N., Ogawa, K., Yonezawa, W., Shii, K. & Baba, S. (1989) *J. Immunol.* **143,** 1155–1162.
63. Chatenoud, L., Thervet, E., Primo, J. & Bach, J. F. (1994) *Proc. Natl. Acad. Sci. USA* **91,** 123–127.