Pancreatic Islet β Cells Drive T Cell–immune Responses in the Nonobese Diabetic Mouse Model

By Etienne Larger, Chantal Bécourt, Jean François Bach, and Christian Boitard

From Institut National de la Recherche Médicale U 25 and Service d'Immunologie Clinique, Hôpital Necker, F-75743 Paris Cedex 15, France

Summary

The role of autoantigens and that of target organs in which tissue lesions develop remains elusive in most spontaneous models of autoimmune diseases. Whether the presence of target autoantigens is required for the recruitment of autoreactive lymphocytes is unknown in most cases. To evaluate the importance of islet cells in the development of autoimmunity in the nonobese diabetic (NOD) mouse, we generated β cell–deprived mice by injecting a high dose of alloxan, a toxic agent specific for β cells. In contrast with spleen cells from 6-mo-old naive NOD mice which transfer diabetes in irradiated 8-mo-old male recipients, spleen cells from age-matched NOD mice which received a single injection of alloxan at 3 wk of age did not transfer diabetes. With the exception of the ability to transfer diabetes, β cell–deprived NOD mice showed maintained immune competence. Furthermore, sialitis developed with the expected intensity and prevalence in β cell–deprived mice. Already committed "diabetogenic" spleen cells collected from spontaneously diabetic mice also showed a reduced capacity to transfer diabetes after their removal from the diabetic mice and transient "parking" in β cell–deprived mice. Taken together, our data bring evidence that involvement of autoreactive T cells detected by the capacity to transfer diabetes requires the presence of target β cells.

The role of antigens in events responsible for the break down of self tolerance is poorly defined in most spontaneous models of autoimmune diseases. Whether the presence of target autoantigens is required for initiating and maintaining immune reponses responsible for the development of autoimmune diseases is a fundamental question. A related issue is whether antigen presentation and the initial activation of autoreactive lymphocytes takes place at the target organ site or within a distant site. Evidence for both local and distant priming and expansion is inferred from experimentally induced autoimmune models. In models in which autoimmunity is triggered by immunization against syngeneic tissues and antigens in CFA, the priming event is distant from the site in which the target autoantigen is expressed (1, 2). Similarly, in transgenic mice expressing a viral protein on target cells, autoimmunity develops after systemic infection with the complete virus, likely as a collateral effect of T cell stimulation occurring outside the target organ (3, 4). The same hypothesis holds in the case of molecular mimicry due to shared antigenic determinants between autoantigens and external antigens (5–7). However, events taking place at the site of target tissues, such as abnormal antigen expression or the local production of cytokines, have been evidenced as initiating autoimmunity in other models (8–11).

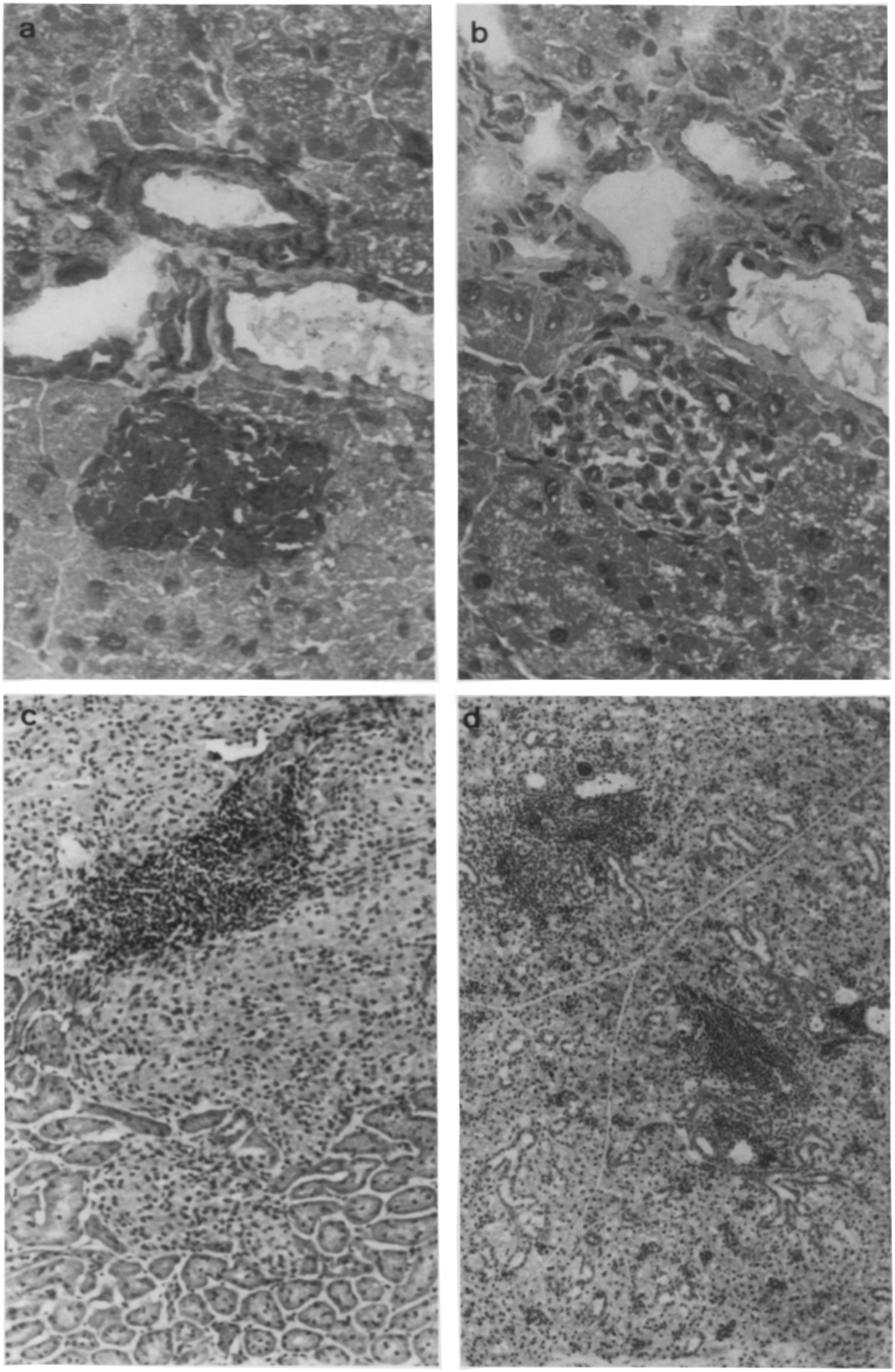
The nonobese diabetic (NOD)[1] mouse is a relevant model to address the role of autoantigen in the development of spontaneous autoimmune diseases. The NOD mouse develops insulitis and diabetes along with other organ infiltrations, particularly sialitis (12, 13). The predominant role of T cells in this model is indicated by the predominance of T cells within the islet infiltrate (14, 15), by the transfer of diabetes into syngeneic recipients by purified T cells from diabetic and nondiabetic mice older than 4 mo of age (16, 17), and by the prevention of diabetes after the injection of mAbs to CD4 T cells and APCs (18–20).

To evaluate the importance of islet insulin-secreting β cells in the development of autoimmunity in the NOD mouse, we generated β cell–deprived mice in which we studied the differentiation of cells tranferring diabetes in conventional NOD recipients, the extent of sialitis, and the recurrence of insulitis in syngeneic islet grafts. We used β cell–deprived mice as recipients of spleen cells from diabetic animals to evaluate the outcome of cells transferring diabetes in the absence of autoantigen-expressing cells.

Materials and Methods

Mice. NOD mice were bred in our facilities under specific pathogen-free conditions and checked every 6 mo for bacterial, viral, and parasitic infections. The spontaneous incidence of diabetes in our colony is 75% in females and 40% in males at 6 mo of age. Mice were monitored for glucosuria (Glucotest; Boehringer Mannheim, Mannheim, Germany) twice a week. Glucosuric mice were tested for glycemia by retroorbitar sinus venous puncture using

[1] *Abbreviation used in this paper:* NOD, nonobese diabetic.


a
b
c
d

test strips and a colorimetric assay (Haemoglucotest and Reflolux F; Boehringer Mannheim). Diabetes was diagnosed as persistent glycemia >3.5 g/liter.

Alloxan. Alloxan (Sigma Chemical Co., St. Louis, MO) was administered by an injection of 150 mg/kg i.v. at weaning or as stated (21). Diabetic mice were treated by daily subcutaneous lente insulin injection (Ultratard; Novo, Bagsvaerd, Denmark). Insulin dosage was modulated according to weight, ranging from 0.5 to 2 U/d.

Histology. Frozen sections of pancreas 5-μm-thick, with at least five levels separated by 120 μm, were stained with hemalun (Merck, Darmstadt, Germany) and eosin. Immunohistochemistry was performed with streptavidin-biotin-horseradish peroxidase (Amersham International, Amersham, Bucks, UK), and Diaminobenzidine as a substrate (Sigma Chemical Co.) using anti-insulin (monoclonal guinea pig anti-swine; Dako, Glostrup, Denmark) and anti-synaptophysin antibodies (polyclonal rabbit anti-human, Dako) (22). Second antibody was either biotinylated goat anti-guinea pig antibody (Jackson ImmunoResearch Labs., Inc., West Grove, PA) or biotinylated goat anti-rabbit antibody (Jackson ImmunoResearch).

Frozen sections of submandibular glands, 7-μm-thick, with at least five sections per gland separated by 100 μm, were stained with hematein and eosin, and evaluated blindly by two independent observers with a grid ocular. On each section were evaluated the surface of sero-mucous parenchyma, the number of infiltrates, and the individual surface of the infiltrates. Results were expressed as the number of infiltrates per 100 squares of sero-mucous tissue, and the ratio surface of infiltrate divided by the surface of sero-mucous parenchyma × 100. Concordance of independent readings was estimated by the interclass correlation coefficient and was found to be >0.94.

Adoptive Transfer of Diabetes. Diabetes was transferred as described by Wicker et al. (16). 8-wk-old male NOD recipients were first irradiated (750 rad) and then injected intravenously with 10^7 spleen cells prepared aseptically in HBSS collected from 6-mo-old alloxan-treated or control mice.

Cotransfer of Diabetes. Cotransfer of diabetes was performed as previously described (23). 8-wk-old, irradiated, male NOD recipients were injected intravenously with 2×10^7 spleen cells collected from 6-mo-old alloxan-treated mice, and 24 h later, with 10^7 spleen cells collected from diabetic NOD mice.

Serial Transfer. Splenocytes from diabetic NOD females were transferred into irradiated, 8-wk-old, normal (controls) or β cell-deprived NOD males (first order recipients). 14 d later, first order recipients served as donors of spleen cells for a second order transfer into similar preirradiated normal or β cell-deprived NOD males. At each transfer step, spleen cells from control or β cell-deprived recipients were tested for their capacity to transfer diabetes into conventional, preirradiated, non β cell-deprived male NOD recipients, as referred to above (adoptive transfer of diabetes).

Anti-human Insulin Antibodies. Anti-human insulin antibodies were detected as described by Palmer et al. (24), by precipitation of iodinated tracer insulin. Results were expressed as percent precipitation of the tracer.

GVHD. (NOD×C57BL/6)F_1 newborn mice received an intraperitoneal injection of 10^7 splenocytes from β cell-deprived NOD mice. Recipients were killed 10 d later. Spleen weight ratio to body weight was measured, and a spleen enlargement index was calculated by dividing the relative spleen weight of experimental animals by the relative spleen weight of littermate controls. The index was considered positive above 1.3 (25).

Islets Grafting. Islets of Langerhans were isolated from 6-wk-old NOD male mice as described by Lacy and Kostianovsky (26) with modifications. Briefly, islets were digested for 6 min in 4 mg/ml collagenase P (Boehringer Mannheim) in PBS, by shaking at 37°C, and washed in PBS. They were then poured on a discontinuous Ficoll gradient (Sigma Chemical Co.). After centrifugation for 20 min at 800 *g*, islets were collected, washed in PBS, and further purified by hand-picking under a microscope. Islets were cultured for 7 d under 5% CO_2 at 37°C in MEM (Gibco, Paisley, UK), 5.5 mM glucose, supplemented with 5% FCS, penicillin, and streptomycin. Mice were anesthesized with Avertin (2.2.2-Tribromomethanol; Aldrich-Chimie, Strasbourg, France). Islets were grafted under the left kidney capsule as described by Ricordi and Lacy (27).

Statistical Analysis. Statistical analysis was performed using Student's *t* test and χ^2 analysis.

Results

Characterization of β Cell-deprived NOD Mice. Female NOD mice deprived of β cells were prepared by performing a single injection of 150 mg/kg of alloxan at the age of 3 wk, i.e., before any detection of islet infiltration by macrophages and T cells. These mice were first characterized as for the absence of β cells. They developed severe diabetes within 24 h after alloxan injection and were kept alive by a daily subcutaneous insulin injection. On histological sections of pancreata collected at 6 mo of age, islets were scarce or replaced by extensive scars. Only a few small remnant islets could be detected that contained cells staining for synaptophysin, a marker of secretory granules of endocrine cells (mostly glucagon and somatostatin), but no insulin-containing cells were detected. However, few sites of inflammatory cells were detected in the vascular spaces and in the vicinity of remnant islets. No invasive infiltration with lymphocytes within remant islets was seen (Fig. 1).

To first evaluate the importance of β cells in allowing the expansion of "diabetogenic" T cells defined by their capacity to transfer diabetes into syngeneic recipients, we tested the capacity of spleen cells from 6-mo-old β cell-deprived donors to transfer diabetes. As shown in Fig. 2, spleen cells from β cell-deprived NOD mice failed to transfer diabetes. None of the preirradiated male recipients had developed diabetes by 12 wk after transfer, as opposed to 89% of recipients of spleen cells from untreated, age-matched, controls. Effector T cells detected by diabetes transfer thus did not differentiate or expand enough to transfer diabetes in the absence of β cells.

As alloxan-mediated massive release of pancreatic β cell antigens at the age of 3 wk in NOD female mice might in-

Figure 1. Islet and salivary gland histology in alloxan-treated NOD mice. (*a–c*) Pancreas and salivary gland histology in 6-mo-old NOD females that received a single injection of 150 mg/kg alloxan at 3 wk of age. (*a* and *b*) the same islet of Langerhans on serial sections stained with (*a*) antisynaptophysin and (*b*) anti-insulin (original ×128). (*c*) Islet graft infiltration 14 wk after grafting (hematoxylin-eosin staining, original ×128). (*d*) Sialadenitis (hematoxylin-eosin staining, original ×51).

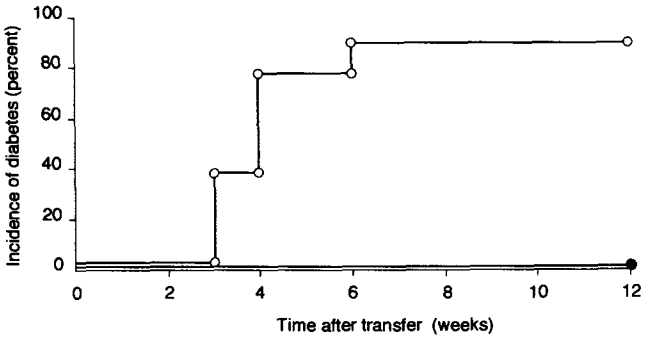


Figure 2. Incidence of adoptive transfer of diabetes from alloxan-treated mice. Nondiabetic and β cell–deprived donors were injected with buffer (PBS) or alloxan (150 mg/kg) as a single intravenous injection at 3 wk of age. 8-wk-old, preirradiated (750 rad), male recipients received an intravenous infusion of 10^7 spleen cells from naive nondiabetic (○, n = 29) or β cell–deprived (●, n = 18) 6-mo-old female donors.

duce regulatory T cells, a cotransfer experiment was performed to evaluate the induction of regulatory T cells in β cell–deprived mice. The transfer of diabetes by spleen cells from spontaneously diabetic animals was actually not inhibited by the cotransfer of spleen cells from 6 mo-old mice pretreated with alloxan at the age of 3 wk (Fig. 3). It is thus unlikely that alloxan induced active suppression when injected in 3 wk-old NOD female mice.

The competence of the immune system of β cell–deprived, insulin-treated animals was evaluated. The evaluation of lymphocyte subsets showed no significant change in alloxan-treated mice. The percentage of B, T, $CD4^+$, and $CD8^+$ cells was respectively 31.7 ± 4.4, 52.3 ± 8.9, 36.9 ± 4.5, and 8.8 ± 3.6 (n = 18) in alloxan-treated mice; 31.3 ± 4.3, 46.0 ± 8.5, 30.6 ± 3.6, and 12.1 ± 3.9 (n = 7) in control mice at 6 mo of age. No significant difference in the absolute number of the various lymphocyte subsets was seen in these mice. We investigated the ability of mice to respond to a foreign protein. At the age of 6 mo, all insulin-treated diabetic mice had high levels of anti-human insulin antibodies: 32.9 ± 14.4% (n = 6) precipitation of radiolabeled insulin tracer, versus 51.3 ± 7.5% (n = 6) for spontaneously diabetic NOD mice treated by insulin for 6 wk. Three β cell–deprived mice were also evaluated by testing the ability of their splenocytes to develop GVH response in (NOD×C57BL/6)F_1 recipients. 10 d after the injection of NOD splenocytes in F_1 neonates, spleen index ratios were comparable to controls: 3.7, 3.8, and 4.0 for β cell–deprived animals, and 4.1 for an untreated diabetic female.

Sialitis in β Cell–deprived NOD Mice. A remarkable, although still unexplained, feature of the NOD mouse disease is, beyond the development of anti-β cell autoimmunity, that of other target tissue infiltrates. We took advantage of β cell–deprived NOD mice to ascertain the role of islet cells in the development of sialitis within salivary glands. Sialitis developed in all β cell–deprived mice to an extent that was comparable to that of control animals (Table 1). The intensity of sialitis suggests that islet cells are not required in the development of sialitis and brings further evidence that immune competence is maintained in β cell–deprived NOD mice.

Serial Transfer of Diabetogenic Spleen Cells in β Cell–deprived Recipients. To ascertain the importance of persisting islet β cells in maintaining T cells from diabetic animals in a diabetogenic functional state, T cells from diabetic mice were removed from their diabetogenic environment and "parked" in β cell–deprived adoptive hosts before their transfer in conventional recipients. We thus performed serial adoptive transfers of spleen cells from spontaneously diabetic donors into irradiated 8-wk-old male, control or β cell–deprived hosts.

After one and two consecutive 14-d passages in control or β cell–deprived recipients, spleen cells were transferred into conventional, preirradiated recipients to evaluate their functional state (Fig. 4). The incidence of diabetes transfer by spleen cells from spontaneously diabetic donors was 75%. When the same spleen cells from diabetic donors were parked once

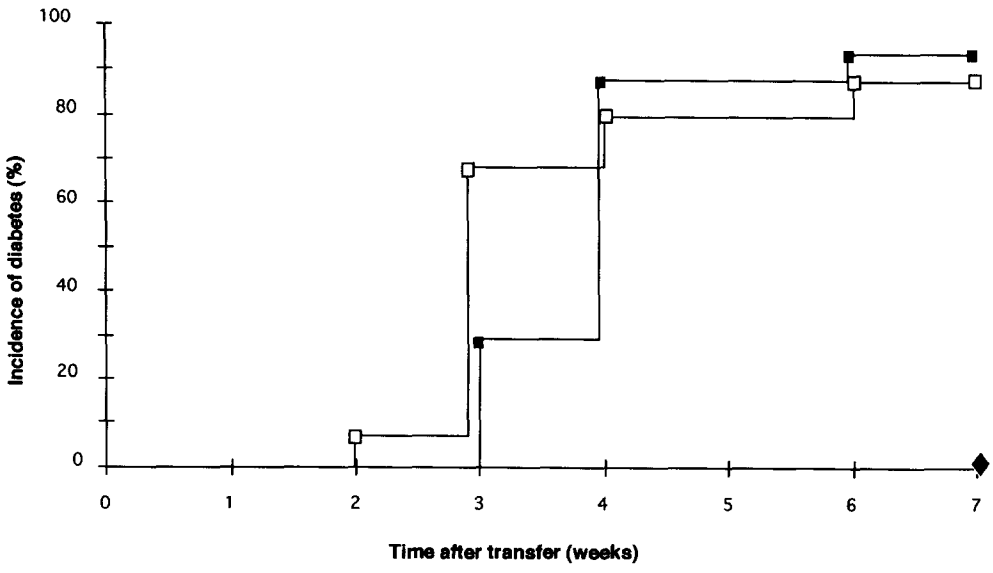


Figure 3. Cotransfer of diabetes. Incidence of diabetes in irradiated (750 rad) 8 wk-old male NOD mice used as recipients of 2 × 10^7 spleen cells from 6-mo-old β cell–deprived NOD mice that received 150 mg/kg alloxan at 3 wk of age, plus 10^7 spleen cells collected from spontaneously diabetic mice (*open symbols*, n = 14), 10^7 spleen cells from spontaneously diabetic mice alone (*closed symbols*, n = 15), and 10^7 spleen cells from β cell–deprived mice alone (*diamonds*, n = 8).

Table 1. *Sialadenitis in NOD Mice at the Age of 6 Mo*

	Number	Surface
Alloxan (n = 6)	1.6 ± 0.6	3.8 ± 2.4
Controls (n = 4)	1.2 ± 0.4*	1.8 ± 0.8*

Results are expressed as number of mononuclear cell infiltrates per 100 squares of serous tissue (Number) and surface of infiltrate divided by the surface of serous tissue × 100 (Surface). Results are expressed as mean ± SD.

* p = Nonsignificant vs alloxan (Student's t test).

or twice for 14 d in preirradiated 8-wk-old controls with a normal β cell mass, the efficiency of transfer was maintained (62.5 and 60%, respectively). In contrast, when spleen cells were parked into irradiated, alloxan-treated, β cell–deprived 8-wk-old hosts, the efficiency of transfer in conventional recipients fell to 40% after one passage and to zero after the second passage (Fig. 4). A toxic effect of alloxan was not responsible for the decreased transfer efficiency, as demonstrated by the 100% prevalence of diabetes in recipients of spleen cells from spontaneously diabetic NOD mice that had received a single injection of 150 mg/kg of alloxan 8 d before transfer. Similarly, insulin treatment or hyperglycemia did not reduce the efficiency of transfer when spleen cells from diabetic donors were parked into spontaneously diabetic, irradiated, NOD recipients treated or not treated, respectively with insulin for the same duration (data not shown).

Recurrence of Insulitis on Syngeneic Islet Grafts. The importance of β cells in allowing the development and the maintainance of diabetogenic T cells does not imply that target β cells are necessary for the initial priming of autoreactive T cells rather than the maintenance of already committed cells in a diabetogenic state. To address this issue, we studied the infiltration of syngeneic islets grafted under the kidney capsule of β cell–deprived NOD mice.

Precultured syngeneic islets were grafted under the kidney capsule of six 6-mo-old NOD mice treated with alloxan at 3 wk of age. Normoglycemia was achieved 48 h after grafting. Five mice were still normoglycemic 14 wk later. The grafts were, however, heavily infiltrated by lymphocytes (Fig. 1). One mouse had an early (7 wk) nonimmune-mediated graft failure. The absence of diabetes 14 wk after islet grafting, and the slow recurrence of insulitis in grafted islets suggest that the whole autoimmune process had to start de novo after grafting, indicating that autoreactive T cells remained unprimed after early alloxan treatment, independently of the development of sialadenitis and exocrine pancreatic infiltration. This is in contrast with the outcome of grafts in three spontaneously diabetic control mice in which diabetes recurred within 4 to 7 d.

Discussion

Following the pioneering demonstration that immunological self-tolerance is an acquired phenomenon (28, 29), evidence has accumulated for the direct role of antigens in eliciting and maintaining immunological tolerance. The presence of antigen is central in driving both deletional and nondeletional mechanisms of tolerance (30–35). The absence or the loss of autoantigen may lead to deficient self-tolerance in vivo (36–38). In autoimmune diseases however, the need for the presence and presentation of self-antigens in mechanisms allowing the breakdown of self-tolerance and the activation and expansion of autoreactive T cells remains unclear. There has been scarce evidence suggesting that autoimmune diseases may directly follow primary immune abnormalities and develop independently of the presentation of autoantigen. Intrinsic immune defects occurring independently of target autoantigens are directly responsible for autoimmune diseases

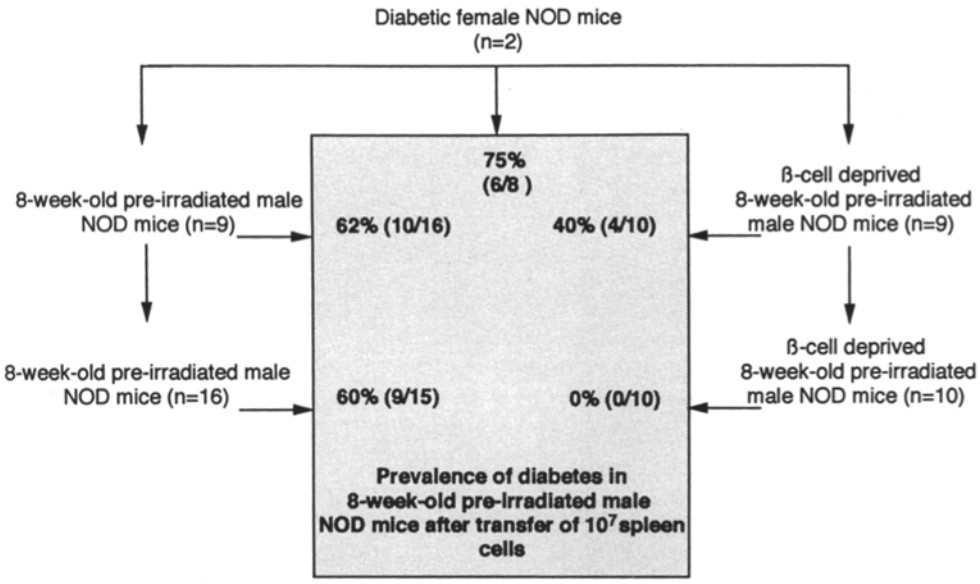


Figure 4. Serial adoptive transfer of diabetes. Prevalence of diabetes in recipients of spleen cells. Ten million splenocytes from spontaneously diabetic NOD mice were transferred into preirradiated (750 rad) 8-wk-old male NOD mice, treated or not by a single intravenous injection of alloxan 7 d before. A second round of adoptive transfer of splenocytes was performed in the same conditions 14 d later. The prevalence of diabetes was studied, at all stages, in preirradiated 8-wk-old male recipients of splenocytes.

observed in immunoproliferative disorders in humans (39). Susceptibility genes may directly alter major immune function as in systemic lupus syndromes associated with the lpr or the gld mutations in the mouse, and in recently developed transgenic mice expressing autoreactive B or T cell antigen receptors or Bcl-2 (40–44). In diabetes, as in most spontaneous autoimmune diseases, it is likely that intrinsic immune defects related to the selection or expansion of the B or T cell repertoire or with abnormalities of immune regulation (45) are only part of a multifactorial process that also involves environmental triggering events and possibly target tissues. Whether the first activation of autoreactive lymphocytes involves the direct recognition of target cell autoantigens or that of antigens sharing cross-reactive epitopes with target antigens remains an open issue. Both cases have been made in transgenic mice in which diabetes has followed the systemic presentation of virus expressing an antigen expressed on β cells as a transgene (3, 4) or the activation of autoreactive T cells by local production of cytokines after targeted cytokine transgene expression (9–11).

Our data point to the requirement for the presence of β cells in the activation of islet-specific T cells in the NOD mouse. After a single injection of a high dose of alloxan before any detectable infiltration of the islets by macrophages and lymphocytes, NOD mice showed no expansion of diabetogenic T cells as evidenced by the failure of spleen cells from 6 mo old, β cell–deprived, NOD mice to transfer diabetes in irradiated recipients. Spleen cells from alloxan-treated mice did not suppress the transfer of diabetes by spleen cells from spontaneously diabetic animals, as indicated by the cotransfer experiment. Tolerized T cells able to downregulate diabetogenic T cells were thus not detectable in alloxan-treated animals. In contrast with established models of immune β cell destruction after repeated injections of low doses of streptozotocin, alloxan has never been reported to induce insulitis, including in the case of repeated low-dose injections (46). Massive doses were chosen to preclude remissions of diabetes which have been reported to occur when two- to threefold lower doses were used (21). Islets were actually scarcely seen on pancreatic sections from β cell–deprived mice, and no β cells were detected in remnant islets. Importantly, sialitis developed with the expected intensity and prevalence in alloxan-treated mice, indicating that immune responses directed against non β-cell related tissues were maintained in alloxan-treated mice. This also indicates that sialitis develops independently of β cells. The absence of detectable expansion of β cell–reactive T cells in alloxan-treated mice brings direct evidence that autoantigens that trigger the anti-islet immune reaction are not expressed or presented in salivary glands, as previously supported by a dissociation of insulitis and sialitis development in transgenic mice (47).

Serial transfer experiments in which spleen cells from diabetic animals were parked in irradiated control or alloxan-treated, β cell–deprived, transient recipients also indicates that the presence of β cells was required to maintain diabetogenic T cells in a functional state allowing the transfer of diabetes. Sublethal irradiation of transient recipients was used to create space in lymphoid tissues for transferred diabetogenic T cells. The kinetics of loss of the capacity to transfer diabetes was somewhat similar to that of memory helper T cells from rats primed with the DNP-KLH hapten–carrier complex in the absence of antigen (48). The recurrence of insulitis within syngeneic islets grafted under the kidney capsule of β cell–deprived mice indicates that the absence of β cell did not induce resistance to insulitis once islet antigens were reintroduced. However, the slow recurrence of insulitis within grafted islets suggests that the whole autoimmune reaction developed as a slow process after grafting as that observed spontaneously in the NOD strain.

Similar observations have been reported in obese strain chickens at the level of B cell activation. The production of antithyroglobulin autoantibodies is not observed in thyroidectomized animals and requires immunization with exogenous thyroglobulin to be triggered (49). The functional status of the thyroid or of the islets of Langerhans is critical in the development of thyroiditis and diabetes, respectively. The dietary iodine content modulates thyroiditis in obese strain chickens (50). Glucose or exogenous insulin reduce as well the incidence of diabetes in the BB rat and in the NOD mouse, possibly relating to altered islet function or antigen expression (51–53). Our data bring direct evidence for the requirement of target cells for a T cell–mediated autoimmune process to develop in vivo and show that β cells themselves drive the anti-β cell T cell immune reponse specifically and independently of the other affected organs.

We wish to thank Miss H. Cohen and Mrs. M. Calisse for care of the animals, and A. Bendelac, P. Matzinger, and C. Carnaud for critical reading of the manuscript.

This work was supported by grants from INSERM.

Address correspondence to Dr. E. Larger, INSERM U 25, Hôpital Necker, 161 rue de Sèvres, F-75743 Paris Cedex 15, France.

Received for publication 5 May 1994 and in revised form 16 December 1994.

References

1. Rose, N.R., and E. Witebsky. 1956. Studies on organ specificity: V. Changes in the thyroid glands of rabbits following acute immunization with rabbit thyroid extracts. *J. Immunol.* 76:417–427.
2. Smith, S.C., and P.M. Allen. 1992. Expression of myosin-class II major histocompatibility complexes in the normal myocardium occurs before induction of autoimmune myocarditis. *Proc. Natl. Acad. Sci. USA.* 89:9131–9135.
3. Ohashi, P.S., S. Oehen, K. Buerki, H. Pircher, C.T. Ohashi, B. Odermatt, B. Malissen, R.M. Zinkernagel, and H. Hengartner. 1991. Ablation of "tolerance" and induction of diabetes by virus infection in viral antigen transgenic mice. *Cell.* 65:305–317.
4. Oldstone, M.B.A., M. Nerenberg, P. Southern, J. Price, and H. Lewicki. 1991. Virus infection triggers insulin-dependent diabetes mellitus in a transgenic model: role of anti-self (virus) immune response. *Cell.* 65:319–331.
5. Oldstone, M.B.A. 1987. Molecular mimicry and autoimmune disease. *Cell.* 50:819–820.
6. Bronze, M.S., E.H. Beachey, and J.M. Dale. 1988. Protective and heart-crossreactive epitopes located within the NH_2 terminus of type 19 streptococcal M protein. *J. Exp. Med.* 167: 1849–1859.
7. Dale, J.M., and E.H. Beachey. 1986. Sequences of myosin-crossreactive epitopes of streptococcal M proteins. *J. Exp. Med.* 164:1785–1790.
8. Sinha, A.A., M.T. Lopez, and H.O. McDevitt. 1990. Autoimmune diseases: the failure of self tolerance. *Science (Wash. DC).* 248:1380–1388.
9. Sarvetnick, N., D. Liggitt, S.L. Pitts, S.E. Hansen, and T.A. Stewart. 1988. Insulin-dependent diabetes mellitus induced in transgenic mice by ectopic expression of class II MHC and interferon-gamma. *Cell.* 52:773–782.
10. Sarvetnick, N., J. Shiruzu, D. Liggitt, L. Martin, B. McIntyre, A. Gregory, T. Parslow, and T. Stewart. 1990. Loss of pancreatic islet tolerance induced by β-cell expression of interferon-gamma. *Nature (Lond.).* 346:844–847.
11. Heath, W.R., J. Allison, M.W. Hoffmann, G. Schönrich, G. Hämmerling, B. Arnold, and J.F.A.P. Miller. 1992. Autoimmune diabetes as a consequence of locally produced interleukin-2. *Nature (Lond.).* 359:547–549.
12. Makino, S., K. Kunimoto, Y. Muraoka, Y. Mizushima, K. Katagiri, and Y. Tochino. 1980. Breeding of a non-obese, diabetic strain of mice. *Exp. Anim.* 29:1–13.
13. Tochino, Y. 1987. The NOD mouse as a model of type 1 diabetes. *CRC Critical Rev. Immunol.* 8:49–81.
14. Miyazaki, A., T. Hanafusa, K. Yamada, J. Miyagawa, H. Fujino-Kurihara, H. Nakajima, K. Nonaka, and S. Tarui. 1985. Predominance of T lymphocytes in pancreatic islets and spleen of prediabetic non obese diabetic (NOD) mice: a longitudinal study. *Clin. Exp. Immunol.* 60:622–630.
15. Signore, A., P. Pozzilli, E.A.M. Gale, D. Andreani, and P.C.L. Beverley. 1989. The natural history of lymphocyte subsets infiltrating the pancreas of NOD mice. *Diabetologia.* 32:282–289.
16. Wicker, L.S., B.J. Miller, and Y. Mullen. 1986. Transfer of autoimmune diabetes mellitus with splenocytes from nonobese diabetic (NOD) mice. *Diabetes.* 35:855–860.
17. Bendelac, A., C. Carnaud, C. Boitard, and J.F. Bach. 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.
18. Shiruzu, J.A., S. Taylor-Edwards, B.A. Banks, A.K. Gregory, and C.G. Fathman. 1988. Immunotherapy of the non obese diabetic mouse: treatment with an antibody to T helper lymphocytes. *Science (Wash. DC).* 240:659–662.
19. Boitard, C., A. Bendelac, M.F. Richard, C. Carnaud, and J.F. Bach. 1988. Prevention of diabetes in non obese diabetic mice by anti-I-A monoclonal antibodies: transfer of protection by splenic cells. *Proc. Natl. Acad. Sci. USA.* 85:9719–9723.
20. Hutchings, P., H. Rosen, L. O'Reilly, E. Simpson, S. Gordon, and A. Cooke. 1990. Transfer of diabetes in mice prevented by blockade of adhesion-promoting receptor on macrophages. *Nature (Lond.).* 348:639–642.
21. Rerup, C.R. 1970. Drugs producing diabetes through damage of the insulin secreting cells. *Pharmacol. Rev.* 22:485–518.
22. Wiedenmann, B., and W.W. Franke. 1985. Identification and localization of synaptophysin, an integral membrane glycoprotein of Mr 38,000 characteristic of presynaptic vesicles. *Cell.* 41:1017–1028.
23. Boitard, C.H., R. Yasunami, M. Dardenne, and J.F. Bach. 1989. T cell–mediated inhibition of the transfer of autoimmune diabetes in NOD mice. *J. Exp. Med.* 169:1669–1680.
24. Palmer, J.P., C.M. Asplin, P. Clemons, K. Lyen, O. Tatpati, P.K. Raghu, and Z.T. Paquette. 1983. Insulin antibodies in insulin-dependent diabetes mellitus before insulin treatment. *Science (Wash. DC).* 222:1337–1339.
25. Simonsen, M. 1962. Graft versus host reactions. Their natural history, and applicability as tools of research. *Progr. Allergy.* 6:349–467.
26. Lacy, P.E., and M. Kostianovsky. 1967. A method for the isolation of intact islets of Langerhans from the rat pancreas. *Diabetes.* 16:35–39.
27. Ricordi, C., and P.E. Lacy. 1987. Renal subcapsular xenotransplantation of purified porcine islets. *Transplantation (Baltimore).* 44:721–723.
28. Owen, R.D. 1945. Immunogenetic consequences of vascular anastomosis bovine cattle twins. *Science (Wash.).* 102:400–401.
29. Billingham, R.E., L. Brent, and P.B. Medawar. 1953. Actively acquired tolerance of foreign cells. *Nature (Lond.).* 172:603–606.
30. Kappler, J.W., N. Roehm, and P.C. Marrack. 1987. T cell tolerance by clonal elimination in the thymus. *Cell.* 49:273–280.
31. Kappler, J.W., U. Staerz, J. White, and P.C. Marrack. 1988. Self-tolerance eliminates T cells specific for Mls-modified products of the major histocompatibility complex. *Nature (Lond.).* 332:35–40.
32. Sha, W.C., C.A. Nelson, R.D. Newberry, D.M. Kranz, J.H. Russell, and D.Y. Loh. 1988. Positive and negative selection of an antigen receptor on T cells in transgenic mice. *Nature (Lond.).* 336:73–76.
33. Nossal, G.J.V. 1989. Immunological tolerance: collaboration between antigen and lymphokines. *Science (Wash. DC).* 245: 147–153.
34. Nikolic-Zugic, J., and M.J. Bevan. 1990. Role of self-peptides in positively selecting the T-cell repertoire. *Nature (Lond.).* 344: 65–67.
35. Von Boehmer, H. 1990. Developmental biology of T cells in T cell-receptor transgenic mice. *Annu. Rev. Immunol.* 8:531–556.
36. Triplett, J. 1962. On the mechanism of immunologic self recognition. *J. Immunol.* 89:505–510.
37. Adams, T.E., S. Alpert, and D. Hanahan. 1987. Non-tolerance and autoantibodies to a transgenic self antigen expressed in

pancreatic β cells. *Nature (Lond.).* 325:223–228.
38. McCullagh, P. 1989. Interception of the development of self tolerance in fetal lambs. *Eur. J. Immunol.* 19:1387–1392.
39. Seligmann, M., and J. C. Brouet. 1973. Antibody activity of human myeloma globulins. *Semin. Hematol.* 10:163–177.
40. Cohen, P.L., and R.A. Eisenberg. 1991. LPR and GLD: single gene models of systemic autoimmunity and lymphoproliferative disease. *Annu. Rev. Immunol.* 9:243–270.
41. Tsao, B.P., K. Ohnishi, H. Cheroutre, B. Mitchell, M. Teitell, P. Mixter, M. Kronenberg, and B.H. Hahn. 1992. Failed self-tolerance and autoimmunity in IgG anti-DNA transgenic mice. *J. Immunol.* 149:350–358.
42. Okamoto, M., M. Murakami, A. Shimizu, S. Ozaki, T. Tsubata, S.-I. Kumagai, and T. Honjo. 1992. A transgenic model of autoimmune hemolytic anemia. *J. Exp. Med.* 175:71–79.
43. Goverman, J., A. Woods, L. Larson, L.P. Weiner, L. Hood, and D. Zaller. 1993. Transgenic mice that express a myelin basic protein-specific T cell receptor develop spontaneous autoimmunity. *Cell.* 72:551–560.
44. Strasser, A., S. Whittingham, D.L. Vaux, M.L. Bath, J.M. Adams, S. Cory, and A.W. Harris. 1991. Enforced BCL2 expression in B-Lymphoid cells prolongs antibody responses and elicits autoimmune disease. *Proc. Natl. Acad. Sci. USA.* 88:8661–8665.
45. Rapoport, M.J., A. Jaramillo, D. Zipris, A.H. Lazarus, D.V. Serreze, E.H. Leiter, P. Cyopick, J.S. Danska, and T.L. Delovitch. 1993. Interleukin 4 reverses T cell proliferation unresponsiveness and prevents the onset of diabetes in nonobese diabetic mice. *J. Exp. Med.* 178:87–99.
46. Rossini, A.A., A.A. Like, W.M. Chick, M.C. Appel, and G.F.J. Cahill. 1977. Studies of streptozotocin-induced insulitis and diabetes. *Proc. Natl. Acad. Sci. USA.* 74:2485–2489.
47. O'Reilly, L.A., P.R. Hutchings, P.R. Crocker, E. Simpson, T. Lund, D. Kioussis, F. Takei, J. Baird, and A. Cooke. 1991. Characterization of pancreatic islet cell infiltrates in NOD mice: effect of cell transfer and transgene expression. *Eur. J. Immunol.* 21:1171–1180.
48. Gray, D., and P. Matzinger. 1991. T cell memory is short-lived in the absence of antigen. *J. Exp. Med.* 174:969–974.
49. Pontes De Carvalho, L.C., T. Jane, G. Wick, and I.M. Roitt. 1982. The role of self antigen in the development of autoimmunity in obese strain chickens with spontaneous autoallergic thyroiditis. *J. Exp. Med.* 155:1255–1265.
50. Brown, T.R., R.S. Sundick, A. Dhar, D. Sheth, and N. Bagchi. 1991. Uptake and metabolism of iodine is crucial for the development of thyroiditis in obese strain chickens. *J. Clin. Invest.* 88:106–111.
51. Gotfredsen, G.F., K. Buschard, and E.K. Frandsen. 1985. Reduction of diabetes incidence of BB Wistar rats by early prophylactic insulin treatment of diabetes prone animals. *Diabetologia.* 28:933–935.
52. Atkinson, M.A., N.K. Maclaren, and R. Luchetta. 1990. Insulitis and diabetes in NOD mice reduced by prophylactic insulin therapy. *Diabetes.* 39:933–937.
53. Gottlieb, P.A., E.S. Handler, M.C. Appel, D.L. Greiner, J.P. Mordes, and A.A. Rossini. 1991. Insulin treatment prevents diabetes mellitus but not thyroiditis in RT6-depleted diabetes resistant BB/Wor rats. *Diabetologia.* 34:296–300.