Rapid Publications

Autoimmune Diabetes in NOD Mouse Is L3T4 T-Lymphocyte Dependent

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SUMMARY

Cultured BALB/c islets fail to function when transplanted into diabetic nonobese diabetic (NOD) mice; such grafted tissue is rapidly destroyed by disease recurrence. The cellular requirements for this graft damage are unclear. This study was designed to investigate the role of the L3T4$^+$ T-lymphocyte subset in disease recurrence in the NOD mouse. L3T4$^+$ T-lymphocytes were depleted by the in vivo administration of the L3T4-specific monoclonal antibody GK1.5. This treatment reduced the level of L3T4$^+$ T-lymphocytes from an initial 43% of the peripheral blood lymphocytes to <4%. L3T4 levels remained at this low level for ~2 wk after withdrawal of GK1.5 treatment, after which the L3T4 levels slowly began to increase in the periphery. Grafting of cultured BALB/c islet tissue into GK1.5-treated diabetic NOD mice resulted in a rapid return to normoglycemia that persisted for 2–4 wk. The gradual return to the hyperglycemic condition roughly correlated with the reappearance of L3T4$^+$ T-lymphocytes in the peripheral circulation. From these findings we conclude that the disease process in the NOD mouse is L3T4 T-lymphocyte dependent. *Diabetes* 36:535–38, 1987

Spontaneous diabetes in rodents such as the BioBreeding (BB) rat or nonobese diabetic (NOD) mouse has an autoimmune etiology and is thought to be a T-lymphocyte–dependent process (1–3). Our laboratory has used islet grafting to analyze this T-lymphocyte involvement in the generation of autoimmune islet damage. The immunogenicity of islet tissue can be eliminated by organ culture in an oxygen-rich atmosphere before grafting (5). When cultured BALB/c islet and pituitary tissues were simultaneously grafted to spontaneously diabetic NOD mice, the islet tissue was destroyed, but the pituitary remained intact (4). That is, islet destruction is a tissue-specific process resulting from disease recurrence in the graft. Induction of allograft immunity resulted in rapid destruction of the cultured pituitary graft (4).

We and others have argued that if islet damage due to disease recurrence results from a direct interaction of the T-lymphocyte with β-cells, then autoimmune damage to grafted islet tissue will be restricted by the major histocompatibility complex (MHC) antigens of the recipient animal (4,6,7). This is because the specificity of antigen-reactive T-lymphocytes is defined by both the nominal antigen and by the MHC antigens of the responsive animal; i.e., allogeneic islets grafted to diseased animals would be resistant to the expression of the disease process. Studies in our laboratory of BB rats (6) and NOD mice (4) grafted with allogeneic islet tissue showed such grafts to be sensitive to the disease process, confirming a report by Weringer and Like (7). We conclude therefore that disease probably does not result from the direct interaction of T-lymphocytes with sensitive β-cells.

An alternate mechanism involves the autoimmune T-lymphocyte interacting with antigen processed by host antigen-presenting cells initiating an inflammatory response that results in islet damage. Because processed antigen is largely presented in the context of class II MHC molecules, it is expected that the L3T4 T-lymphocyte subset, which is predominantly class II restricted (8,9), would play a crucial role in this process. We tested the hypothesis that the L3T4 T-lymphocyte is required for disease recurrence in the NOD mouse. Results show that the elimination of L3T4 T-lymphocytes from diabetic NOD mice, before grafting with cultured allogeneic (BALB/c) islet tissue, inhibits the development of disease in the target tissue. We therefore conclude that this disease is an L3T4 T-lymphocyte–dependent process in the NOD mouse.

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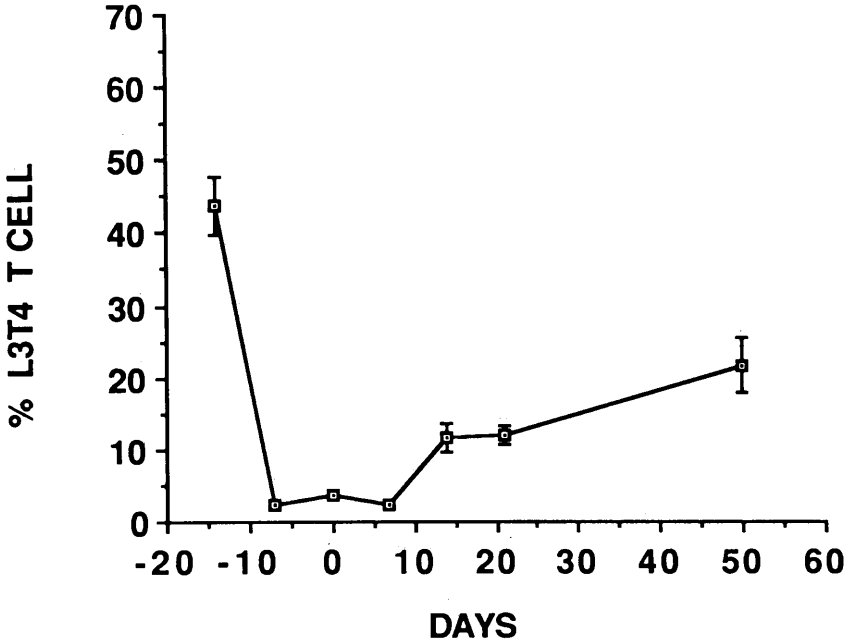

FIG. 1. L3T4 T-lymphocytes as fraction of total lymphocytes in peripheral blood (mean ± SE) in 5 diabetic NOD mice after injection of GK1.5 (200 mg/kg i.p.) on days −14, −7, and 0.

MATERIALS AND METHODS

Animals. NOD/Den mice were derived from a nucleus obtained from Ehime University, Japan, and have been inbred in Denver for seven generations. The incidence of diabetes is ~70% in females and ~30% in males. The nonfasting blood glucose level for nondiabetic NOD/Den mice is 6.2 mM with a 99% confidence interval of ±3.1 mM. BALB/c ByJ mice were obtained from Jackson Laboratories (Bar Harbor, ME).

Management of diabetic NOD mice. Severe diabetes mellitus was diagnosed by glycosuria. Animals used in this study had blood glucose concentrations >20 mM for 3–7 days before treatment. Before grafting, diabetic animals were maintained with 1 U regular insulin (Lilly) · 100 $(mg/dl)^{-1}$ urine glucose · day^{-1} s.c.

After transplantation, blood glucose was measured once a week. Once hyperglycemia returned, the animals were killed, and the grafts were removed for histological examination.

Preparation and grafting of islet tissue. Islet tissue was prepared from the pancreases of BALB/c mice and was cultured for 7 days in 95% O_2/5% CO_2 as previously described (10). Nine clusters of cultured BALB/c islet, each containing 50–60 islets, were transplanted under the kidney capsule of test animals on day 0 (10).

Preparation and administration GK1.5 antibody. NOD mice were treated with the L3T4-reactive rat monoclonal antibody designated GK1.5 (8). Sublethally irradiated (550 rads) BALB/c mice were injected intraperitoneally with pristane (2,6,10,14-tetramethylpentadecane; Sigma T-7640), 0.5

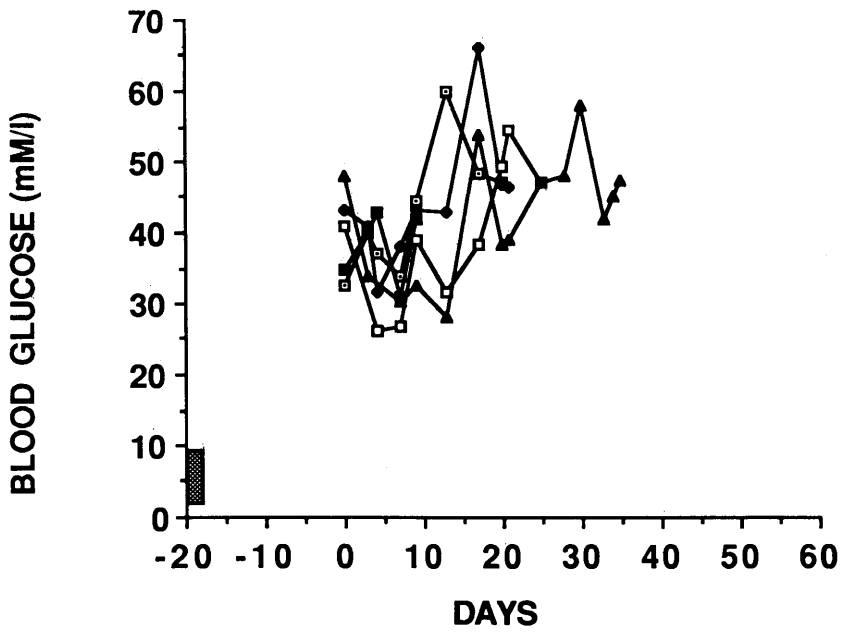

FIG. 2. Blood glucose concentration of 5 diabetic NOD mice transplanted with BALB/c islet tissue on day 0. *Shaded area* shows normal blood glucose range of nondiabetic NOD mice (mean ± 99% confidence interval). Each *symbol* represents 1 animal.

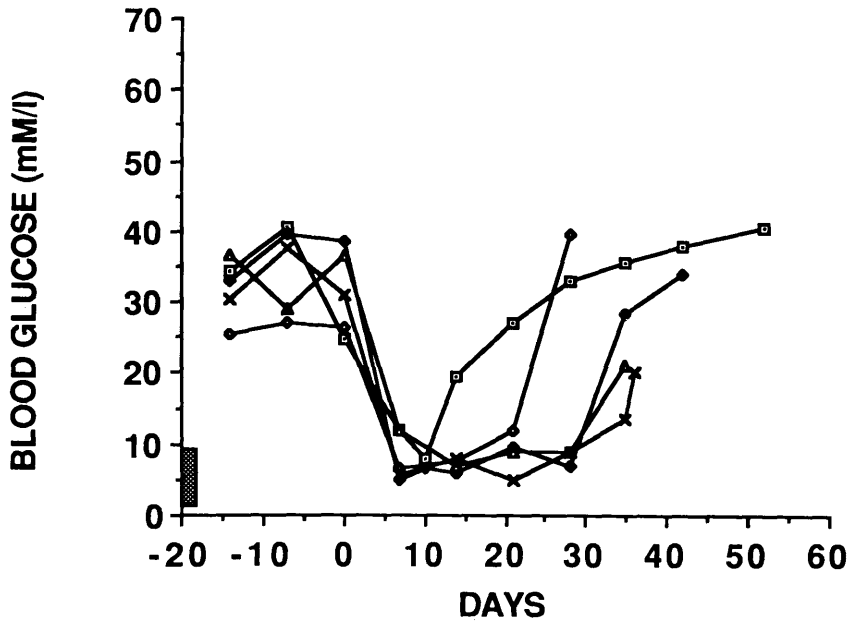

FIG. 3. Blood glucose concentration of 5 diabetic NOD mice transplanted with BALB/c islet tissue on day 0 and treated with GK1.5 on days −14, −7, and 0. *Shaded area* shows normal blood glucose range of nondiabetic NOD mice (mean ± 99% confidence interval). Each *symbol* represents 1 animal.

ml/mouse, 8 days before irradiation. Ten million GK1.5 hybridoma cells were injected intraperitoneally 1 day after irradiation, and ascitic fluid was harvested as it accumulated in the peritoneal cavity. The IgG concentration of pooled ascitic fluid was determined by ELISA as previously described (11).

To study the kinetics of L3T4 T-lymphocyte depletion, five diabetic NOD mice were treated with GK1.5 antibody (200 mg/kg i.p.) on days −14, −7, and 0, and peripheral blood was obtained once a week from individual mice by retroorbital bleeding into heparinized pipettes. Control L3T4 levels were determined from blood samples taken before GK1.5 treatment.

Analysis of L3T4 T-lymphocyte in peripheral blood. The level of L3T4 T-lymphocyte as a percentage of total peripheral lymphocytes was measured by direct-fluorescence analysis as described previously (12). Briefly, heparinized blood aliquots were diluted 1:1 with Hanks' balanced salt solution (HBSS) containing 2% heat-inactivated fetal calf serum (FCS) and 0.15% sodium azide. The samples then were incubated with fluorescein-conjugated GK1.5 monoclonal antibody for 30 min on ice. After lysing the red blood cells with 0.85% ammonium chloride solution, the samples were washed in HBSS-FCS, fixed in a 1% solution of paraformaldehyde in phosphate-buffered saline, and kept in the dark at 4°C. The percent of lymphocytes stained with GK1.5-fluorescein was determined by analyzing 1500 peripheral lymphocytes with an EPICS C flow cytometer (Coulter Electronics, Hialeah, FL). Background fluorescence, determined on an unstained control sample, was subtracted from each sample.

RESULTS

A group of five diabetic NOD mice was treated with GK1.5 antibody (200 mg/kg i.p.) on days −14, −7, and 0. This treatment reduced the level of L3T4 T-lymphocytes in the peripheral blood from the normal levels of ~43% of total lymphocytes to <4% by day 0 (Fig. 1). L3T4 T-lymphocytes remained at this low level for another week. However, by day 14, the level of L3T4 T-lymphocytes showed a significant rise that slowly increased with time. By 50 days after cessation of GK1.5 treatment, the L3T4 T-lymphocyte level was approximately half the level seen in the animals before treatment with GK1.5.

Transplantation of cultured BALB/c islet tissue to spontaneously diabetic NOD mice failed to bring the blood glucose into normal range (Fig. 2). We have previously shown that this inability of islet tissue to function resulted from disease recurrence in the graft tissue (4). However, when the same islet tissue was transplanted to animals treated with GK1.5 antibody, all grafts brought blood glucose to the normal range within a week of transplantation (Fig. 3). Normoglycemia was not maintained indefinitely; grafted animals became hyperglycemic 14–30 days after transplantation (Fig. 3). This return of the disease process was correlated with the reappearance of L3T4 T-lymphocytes in the peripheral blood (Fig. 1). Histological examination of grafted tissue after the return of hyperglycemia showed evidence of severe immunologic damage; in most cases the islet tissue was completely destroyed, with only infiltrating mononuclear cells remaining.

DISCUSSION

The data presented here show that disease recurrence in diabetic NOD mice transplanted with cultured BALB/c islet tissue is an L3T4 T-lymphocyte–dependent process. This conclusion is consistent with previous findings that suggested autoimmune islet damage did not result from a direct attack by T-lymphocytes. The disease process was not restricted by the MHC antigens of the grafted islet tissue, indicating that T-lymphocytes were not interacting with antigens in association with MHC antigens on the β-cell surface (4,6,7). In the rat, it has been suggested that natural killer (NK) cells might be the proximate mediators of islet damage (3,13). This conclusion is consistent with our findings in the mouse, because the activation of NK cells is a lymphokine-

dependent process (14), and L3T4 T-lymphocytes could function as activators of NK cells.

Mandrup-Poulsen et al. (15) have proposed that islet damage is triggered by the local production of interleukin 1 (IL-1) by activated macrophages. Such a process could also be L3T4 T-lymphocyte dependent, because these T-lymphocytes are usually restricted by class II MHC antigens and so can interact with macrophages that present processed antigen. Our suggestion that autoimmune islet destruction resulted from free radical damage (4) to β-cells also is consistent with the cellular interaction described above. One attractive feature of a free radical–mediated mechanism (that could be triggered in the β-cell by IL-1) is that it would provide specificity to an otherwise nonspecific inflammatory response, because the β-cell is more sensitive to free radical damage than are other cells in the pancreatic islet (16).

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