A diabetic syndrome closely resembling human IDDM has been induced in rats of specific pathogen-free origin by a combination of thymectomy and irradiation, with an overall incidence (10 wk postirradiation) in female rats of 34% for acute disease and 47% for islet lesions. Males were slightly more susceptible than females. Clinical features of the syndrome included hyperglycemia, insulinopenia, ketosis, and lipemia, and corresponding islet pathology ranged from diffuse atrophy to focal atrophy and insulitis. Onset was usually acute, and the disease fatal unless early insulin therapy was initiated. Lymphocytic thyroiditis also was observed in a proportion of thymectomized and irradiated rats (49% in females) over the same period but with no apparent correlation to the occurrence of diabetes. A significant decrease in the incidence of disease was found in thymectomized and irradiated rats of conventional origin when compared with genetically identical specific pathogen-free rats, implicating a role for environmental factors in disease susceptibility. Diabetes induction also was found to be strain related but not RT1<sup>L</sup>-dependent. Both clinical signs and islet lesions were inhibited by early reconstitution of thymectomized and irradiated animals with syngeneic lymphoid cells from normal donors. Islet lesions and glucose intolerance could be transferred to syngeneic recipients by concanavalin A–activated lymphoid cells from acute diabetic donors. The close similarities between this experimental syndrome induced by immunological manipulation and the clinical condition in humans provide further evidence for an immune-mediated pathogenesis for IDDM. Diabetes 42:571–78, 1993

Experimental animal models of IDDM provide opportunities to investigate the many interacting factors contributing to disease initiation and pathogenesis that are not possible in humans. Such experimental animal studies have already led to many important findings and may eventually enable rational strategies for disease prevention and control to be developed (1).

Diabetic syndromes occur spontaneously in numerous animal species (2) and of these, the BB rat and NOD mouse have been extensively characterized (3,4). Diabetes has been induced experimentally in animals with various chemical agents or hormones (5), and chemical agents have been used to enhance or inhibit diabetes expression in spontaneous models (6–9). In addition, viral agents have been shown to induce diabetes in animals not predisposed to disease induction (10) and to modify disease expression in NOD mice (11,12) and BB rats (13). Together, these induced models have provided much useful information, particularly with regard to the influence of environmental agents on disease pathogenesis. To date however, no well-characterized induced model of human IDDM has been available that does not rely on chemical or infectious agents for disease expression. The availability of such a model would offer an adjunct to spontaneous models for the study of disease pathogenesis while avoiding possible adverse secondary effects of chemical or viral agents.

This study describes the features of a diabetic syndrome induced in rats by manipulation of the immune system with a combination of thymectomy and irradiation, Tx-X. This induced syndrome appears to be closely analogous to the clinical situation in humans and has proved readily amenable to procedures that can modify
expression of disease. It thus has potential value as an experimental model for studies of diabetes initiation and pathogenesis.

RESEARCH DESIGN AND METHODS
We obtained 21-day-old SPF inbred female or male PVG (RT1<sup>C</sup>) and female WAG (RT1<sup>U</sup>), Lou/m (RT1<sup>U</sup>), SHR (RT1<sup>U</sup>), DA (RT1<sup>U</sup>), and F344 (RT1<sup>U</sup>) strain rats from the Animal Resources Centre in western Australia and housed them under nonbarrier conditions in the Murdoch University animal house. Inbred PVG rats of conventional origin (i.e., non-SPF) were bred from our own stock and were maintained in an adjacent room. All PVG animals, whether SPF or conventional, were derived from the same parental stock. The 21-day-old F1 generation RT1<sup>CvU</sup> hybrid rats were derived from matings between SPF PVG (RT1<sup>C</sup>) female and WF (RT1<sup>U</sup>) male rats. Rats were maintained in groups of 3–5/cage and fed on standard pelleted rat feed with water provided ad libitum. Unless otherwise indicated, we used female SPF-derived PVG inbred animals for all experiments.

**Tx-X protocol and disease monitoring.** Early-age thymectomy followed by low-dose whole-body irradiation was performed as described previously (14). Briefly, 3-wk-old rats were thymectomized under sodium pentobarbital anesthesia in groups of 20–30 at one time, given four fortnightly doses of whole-body 7-irradiation (2.5 Gray) and maintained for 8–10 wk, during which time diabetes developed spontaneously in some animals (Fig. 1). During the 8- to 10-wk development period after final irradiation, animals were examined daily for clinical signs of diabetes, detectable by a rapid change in overall condition and behavior. Affected animals were tested for hyperglycemia (Glucostix/Glucometer, Bayer Diagnostics, Mulgrave, Australia) by using caudal vein blood samples taken under ether anesthesia, and classified as diabetic on the basis of a blood glucose reading of >13.5 mM. Some clinically diabetic animals were treated daily with subcutaneous doses of human insulin (1.5 U each of Protaphane/Actrapid) (CSL Novo, Melbourne, Australia) to maintain normal blood glucose levels. Insulin treatment, if required, was continued for the duration of the irradiation period, and animals were reconstituted with single spleen cell suspensions from acute diabetic donors every 2 mo, and rechallenged with subcutaneous insulin injections (16). Briefly, spleens were minced and gently pressed through a stainless steel mesh (0.5 mm<sup>2</sup>), washed three times in 0.1 M PBS and 1 x 10<sup>8</sup> viable cells immediately administered intraperitoneally to Tx-X rats within 3 days of their final irradiation. After 10 wk, reconstituted animals were killed and analyzed as described above.

**Histopathology.** Histopathological change was assessed on formalin-fixed, hematoxylin- and eosin-stained pancreas sections. Islet lesions were classified as either 1) diffuse atrophy, if all islets were affected; 2) focal atrophy, if only a proportion of islets were atrophic; and/or 3) insulitis, if lymphocytic infiltration of islets was observed. Sections were assessed without prior knowledge of clinical status.

**Immunofluorescence.** Formalin-fixed sections of rat pancreas were examined for the presence and distribution of glucagon (α)-synthesizing and insulin (β)-synthesizing cells by immunofluorescence. β-cells were detected with an anti-insulin monoclonal antibody (Amersham, Amersham, UK) followed by an antirabbit IgG FITC conjugate (Miles, Yeda, Israel). Islet cell cytoplasmic antibodies were detected on formalin-fixed, trypsin-digested sections of canine pancreas by using diluted plasma from Tx-X rats or non-Tx-X PVG rats as controls, followed by an antirat IgG FITC conjugate (Silenus).

**Plasma IRI and lipid determinations.** Plasma IRI and lipid levels were analyzed in a proportion of samples from acute diabetic animals (not insulin-treated) or from normal (non-Tx-X) rats. IRI radioimmunoassays were performed by the Department of Medicine, Royal Postgraduate Medical School, London, UK, and acetoacetate and β-hydroxybutyrate levels were determined enzymatically as described previously (15).

**Tests for pathogenic agents.** Serum samples from parent breeding stock were tested routinely (3-mo intervals, n = 60) by the Department of Agriculture, South Australia for antibodies to the following common rodent pathogens: *Mycoplasma pulmonis*, murine hepatitis/rat coronavirus, murine cytomegalovirus, minute virus of mouse, pneumonia virus of mouse, Revirus type 3, adenovirus, lymphocytic choriomeningitis, mouse encephalomyelitis, encephalomyocarditis, Sendai, Kilham’s rat, Toolan HI, and Hantaan viruses. A small group of parent breeding stock (n = 6) were randomly selected every 2 mo, and pharyngeal and caecal samples were examined for the presence of a range of recognized bacterial and parasitic pathogens (*Pasteurella* sp., *Yersinia* sp., *Bordetella* sp., *Streptococci* sp., *Listeria* sp., *Salmonella* sp., *Streptobacillus moniliformis*, *Hexamita*, and pinworm). In addition, a smaller number of samples from SPF or conventional Tx-X rats also were tested. All samples were found to be negative on all occasions except one Tx-X, SPF-derived, nondiabetic sample, which was weakly positive for antibody to *M. pulmonis*. Larval stages of the liver parasite *Taenia taeniaeformis* were occasionally isolated from conventionally derived animals.

**Lymphocytic reconstitution of Tx-X rats.** Tx-X rats were reconstituted with single spleen cell suspensions from syngeneic PVG donors as described previously (16). Briefly, spleens were minced and gently pressed through a stainless steel mesh (0.5 mm<sup>2</sup>), washed three times in 0.1 M PBS and 1 x 10<sup>6</sup> viable cells immediately administered intraperitoneally to Tx-X rats within 3 days of their final irradiation. After 10 wk, reconstituted animals were killed and analyzed as described above.

**Adoptive transfer of disease.** Spleen cell suspensions prepared from acute diabetic donors were depleted of
erythrocytes by NH4Cl lysis and cultured at a concentration of 2 x 10^6/ml for 72 h in RPMI containing FCS (10%), glutamine (2 mM), penicillin (100 U/ml), streptomycin (100 ug/ml), 2-mercaptoethanol (0.05 mM), and ConA (Sigma, St. Louis, MO) at 2.5 u/g/ml in 7% CO2 at 37°C. After harvesting, cells were washed three times in RPMI, resuspended to 0.5-1 x 10^7 viable cells/ml in PBS and transferred intravenously to either 3- or 6-wk-old normal female PVG rats, irradiated adult female PVG rats, or nondiabetic Tx-X rats 8–9 wk after final irradiation. Recipients were killed 10 days after transfer, and blood samples and pancreases were taken for analysis. Intraperitoneal glucose tolerance tests were performed on nondiabetic Tx-X rats at 8–9 wk postirradiation to select appropriate recipients for the above cell transfer and also after transfer to test for abnormal glucose regulation as follows: blood glucose levels were determined immediately before intraperitoneal administration of glucose (2 mg/g body weight) and thereafter at 30-, 60-, 120-, and 300-min intervals. Tests were performed at the same time of day on nonfasted animals, and only those showing a blood glucose increase within normal limits were selected as cell-transfer recipients. Recipient animals, together with Tx-X control rats, were retested as above for glucose intolerance 10 days after transfer and necropsied and examined for hyperglycemia and islet lesions. Statistical analysis. Comparison of incidence rates was by y^2 analysis of 2 x 2 contingency tables using Yates' correction for n > 50 or Fisher's Exact Test for n <= 50 (17). Mean values were compared with Student's t test for unpaired samples.

RESULTS

Disease characterization

Onset and incidence of diabetes. Onset of acute diabetes was recognized by a rapid loss of condition and a concurrent increase in blood glucose levels. Initiation of parenteral insulin therapy at this point was effective in controlling blood glucose and weight loss and restoring body condition, whereas delay or withdrawal of insulin therapy resulted in further wasting and hyperglycemia, leading to death within 1–4 days of disease onset. Onset of this acute diabetic syndrome occurred with a peak at 4 wk after final irradiation and was rarely observed before study wk 3 or after study wk 8 (Fig. 2). The incidence of acute diabetes in successive Tx-X groups established at 1-mo intervals is shown in Fig. 3. Although acute diabetes occurred regularly, considerable variations in incidence levels were observed, ranging from 11 to 62%. Overall, acute diabetes occurred with a mean incidence of 34% for all Tx-X rats established over this period (n = 392 in 19 separate groups).

Islet histopathology. Hematoxylin and eosin staining of pancreatic sections taken from acute diabetic rats shortly after onset of clinical signs revealed severe lesions usually confined to the islets, although occasionally, pancreatitis was observed. In addition to acute diabetes, a more chronic condition also was identified retrospectively on the basis of islet lesions, and hyperglycemia was detected in samples taken at necropsy. These animals maintained normal body weight and condition throughout the observation period and did not become insulin dependent. A third group of Tx-X rats was normoglycemic but had islet lesions at necropsy. The proportion of Tx-X rats with islet lesions that developed acute diabetes ranged from 100% in some groups to <50% in others. Incidence of islet histopathology in Tx-X rats, whether acute diabetic or otherwise, ranged from 17 to 75% between groups, with an overall mean of 47% for all treated rats (Fig. 3). Acute diabetes or islet lesions were not detected in normal, unmanipulated PVG rats maintained over the same period (n = 25).

Representative islet morphology seen in Tx-X rats is illustrated in Fig. 4. The degree of islet change generally reflected the clinical condition of the animal, ranging from diffuse atrophy (Fig. 4C) in most acute diabetic animals to varying degrees of insulitis and focal atrophy in chronic and nondiabetic Tx-X rats (Fig. 4B). Table 1 summarizes the frequency with which these islet lesion types were observed in Tx-X rats. Diffuse atrophy was confined to the majority of acute animals, consistent with major impairment of β-cell function within this group, and was never observed in chronic or nondiabetic Tx-X rats (P < 0.001). Focal atrophy, usually together with insulitis,
TX-X INDUCED IDDM IN RATS


TABLE 1
Frequency of islet lesions in diabetic or nondiabetic Tx-X rats

<table>
<thead>
<tr>
<th>Lesion classification</th>
<th>Diabetic rats, n (%)</th>
<th>Nondiabetic rats, n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Acute*</td>
<td>Chronic†</td>
</tr>
<tr>
<td>Diffuse atrophy</td>
<td>57 (75)§</td>
<td>0</td>
</tr>
<tr>
<td>Focal atrophy</td>
<td>17 (22)§</td>
<td>3 (38)</td>
</tr>
<tr>
<td>Insulitis</td>
<td>2 (3)§</td>
<td>5 (63)</td>
</tr>
</tbody>
</table>

*Hyperglycemia with insulin dependence.
†Hyperglycemia without insulin dependence.
§P < 0.001 compared with nondiabetic rats.
||P < 0.01 compared with nondiabetic rats.

was commonly observed in diabetic rats, but rarely in nondiabetic animals (P < 0.001 compared with acute diabetic rats). In contrast, acute diabetic animals rarely showed insulitis, but this was the most frequent lesion of chronic and nondiabetic animals (P < 0.01 compared with acute diabetic rats). These observations provide an insight into lymphocyte dynamics associated with islet pathogenesis, indicating that insulitis precedes the development of atrophy, and thereafter, once atrophic changes ensue, infiltrating lymphocytes quickly disappear from the lesion site. Neither islet atrophy nor insulitis was ever observed in control unmanipulated PVG animals (n = 25).

Routine examination of thyroid glands from Tx-X rats also revealed a proportion with lymphocytic thyroiditis. This condition occurred with an overall incidence of 49% in female animals and showed no correlations with acute diabetes.

To investigate whether the islet cell destruction was selective within affected islets, sections of affected tissue were stained by immunofluorescence for α-cells (glucagon-synthesizing) and β-cells (insulin-synthesizing). Atrophic islets failed to stain with the anti-insulin monoclonal antibody, indicating a complete loss of β-cell mass characteristic of end-stage atrophy (Figs. 5A and 5C). In contrast, α-cells were readily detected in atrophic islets and had become the predominant cell type, now in a central location (Figs. 5B and 5D). In nonatrophied but infiltrated islets, β-cells could still be detected by immunofluorescence, however, the cells were dispersed throughout the islet rather than occupying their usual central location (data not shown). These data indicate that β-cells are the primary target for immunological destruction in islets of acute diabetic Tx-X rats.

**Antibodies to islet cell antigens.** Antibodies to islet cell antigens were demonstrated by immunofluorescence in 4 of 16 (25%) serum samples taken from acute diabetic animals at time of disease onset, but the intensity of fluorescence within this group varied considerably.

**Clinical chemistry.** Acute diabetic animals had markedly raised levels of plasma glucose, acetoacetate, and 3-hydroxybutyrate compared with normal levels (P < 0.001) and correspondingly depressed circulating IRI levels (P < 0.001), revealing profound disturbances in glucose regulation and compensatory changes in fat metabolism in these rats (Table 2).

**Factors affecting disease expression**

**Gender.** Incidence levels of diabetes and islet lesions in female as compared with male SPF-derived Tx-X rats are shown in Table 3. Mean levels of diabetes and islet lesions were higher in males compared with females (P < 0.05 for islet lesions), thus suggesting males are slightly more susceptible to diabetes inducement by this procedure.

**Environmental.** To assess the possible influence of environmental factors on Tx-X-induced diabetes, disease expression was compared in rats derived from common parental stock but bred under different hygienic conditions (Table 3). Conventionally bred (non-SPF) rats showed a strikingly reduced susceptibility to diabetes inducement when compared with SPF Tx-X animals.
maintained concurrently, with no conventional animals developing diabetes ($P < 0.001$). A significant reduction in islet lesions was observed in conventional compared with SPF Tx-X rats ($P < 0.025$).

**Genetic.** Genetic influences on the inducement of diabetes by the Tx-X procedure were examined in crossbred animals and numerous inbred SPF-derived rat strains of differing RT1 background. PVG (RT1$^b$)/WF (RT1$^u$) crosses were bred primarily to examine the influence of RT1$^u$ superimposition on the PVG genetic background. Although male crossbred animals showed increased levels of diabetes and islet lesions compared with male inbred animals, these differences were not statistically significant, and no differences were observed for similar female comparisons (Table 4). When inbred rat strains differing at the RT1 locus were compared for disease expression, only WAG strain rats were found to be susceptible to disease inducement (Table 4).

**Lymphocyte transfer studies**

**Adoptive transfer of disease.** Adoptive transfer experiments were summarized in Table 5. Although diabetes was not observed in any recipient, islet lesions were induced in a proportion of normal 3-wk-old and adult irradiated female SPF-derived recipients and in all non-diabetic Tx-X recipients. In addition, all Tx-X recipients displayed abnormal glucose tolerance 10 days after cell transfer compared with a group of nondiabetic Tx-X control rats. Tx-X rats were more susceptible to transfer presumably as a consequence of impaired immunoregulation. In view of the ability to transfer lesions and other changes, the failure to transfer diabetes may reflect the relatively low numbers of cells available (0.5–2 x 10$^7$).

**Disease prevention by lymphocyte reconstitution.** The transfer of viable lymphoid cells isolated from normal (non-Tx-X) PVG donors to Tx-X rats shortly after final irradiation was effective in inhibiting the onset of autoimmune disease in these rats (Table 6). Thus, levels of islet lesions ($P < 0.025$) and thyroid lesions ($P < 0.001$) were significantly reduced in reconstituted rats compared with non-reconstituted Tx-X control rats.

**TABLE 2**

<table>
<thead>
<tr>
<th>Glucose (mM)</th>
<th>Acute diabetic rats*</th>
<th>Normal rats (non-Tx-X)</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>7</td>
<td>10.8 ± 0.4</td>
</tr>
<tr>
<td>Insulin (pM)</td>
<td>7</td>
<td>9.4 ± 3.3</td>
</tr>
<tr>
<td>Acetoacetate (mM)</td>
<td>10</td>
<td>0.97 ± 0.3</td>
</tr>
<tr>
<td>β-hydroxybutyrate (mM)</td>
<td>10</td>
<td>9.11 ± 1.5</td>
</tr>
</tbody>
</table>

Data are means ± SE. $P < 0.001$ for all comparisons.

*Hyperglycemia with insulin dependence. Samples were taken at onset of clinical signs.

†Detection limit of assay = 5 pM.

DIABETES, VOL. 42, APRIL 1993
The manner of disease inducement supports the contention that this form of diabetes is immune mediated, and it is unlikely that γ-irradiation per se could have been directly responsible for the islet injury because of the low dosage level used and the long interval between irradiation and diabetes onset. Furthermore, the lymphocytic infiltration of islets, the occurrence of autoantibodies to islet cell components, and the lymphocyte transfer experiments showing that disease pathogenesis is both lymphocyte determined and regulated provide compelling evidence for an immunological basis. In a subsequent study, antibodies to a 64,000-Mr subunit of the enzyme glutamic acid decarboxylase are a consistent feature (18).

Our attempts to induce diabetes in inbred rat strains of differing genetic composition maintained concurrently in the same environment by thymectomy and irradiation revealed differences in susceptibility, providing evidence for a genetic input. Similarly, the spontaneous diabetic BB rat model has an essential genetic requirement (19), and in this case, crossbreeding studies have shown the need for at least one RT1U-bearing haplotype for disease expression, and an abnormality within the RT1U locus may exist in this particular strain (20). It is evident that the RT1U genotype is not essential for diabetes inducement by the Tx-X procedure because PVG rats of RT1c background were most susceptible when compared with other strains investigated, including those possessing RT1U genes. Other studies in rats also have shown that autoimmune diabetes is not exclusively related to the RT1U MHC haplotype. For example, McKeever et al. (21) induced diabetes in PVG background nude recipients by transfer of ConA-activated, RT6-depleted syngeneic lymphocytes derived from PVG rats.

An interesting feature of this model is the slightly higher incidence of disease in males than females. BB rats, on the other hand, have been shown to have an equal incidence of diabetes and islet lesions in female or male Tx-X rats of SPF or conventional origin.

**TABLE 3**

<table>
<thead>
<tr>
<th>Strain</th>
<th>RT1 genotype</th>
<th>n</th>
<th>Diabetes incidence, n (%)</th>
<th>Islet lesions, n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Acute†</td>
<td>Chronic†</td>
</tr>
<tr>
<td>Female</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SPF</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SPF</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Conventional rats</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Hyperglycemic with insulin dependence. †Hyperglycemic without insulin dependence. §Itpatroph or insulitis in both diabetic and nondiabetic rats. $P < 0.05 compared with SPF value.
||| $P < 0.005 compared with SPF female value. || $P < 0.005 compared with SPF female value. || $P < 0.025 compared with SPF value.

**DISCUSSION**

These data extend our previous studies (15) demonstrating that IDDM can be consistently induced in rats not normally prone to the spontaneous development of this disease by a procedure that modulates immune function. The manner of disease inducement supports the contention that this form of diabetes is immune mediated, and it is unlikely that γ-irradiation per se could have been directly responsible for the islet injury because of the low dosage level used and the long interval between irradiation and diabetes onset. Furthermore, the lymphocytic infiltration of islets, the occurrence of autoantibodies to islet cell components, and the lymphocyte transfer experiments showing that disease pathogenesis is both lymphocyte determined and regulated provide compelling evidence for an immunological basis. In a subsequent study, antibodies to a 64,000-Mr subunit of the enzyme glutamic acid decarboxylase are a consistent feature (18).

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An interesting feature of this model is the slightly higher incidence of disease in males than females. BB rats, on the other hand, have been shown to have an equal

**TABLE 4**

Incidence of diabetes and islet lesions in Tx-X rats of differing genetic background

<table>
<thead>
<tr>
<th>Strain</th>
<th>RT1 genotype</th>
<th>n</th>
<th>Diabetes incidence, n (%)</th>
<th>Islet lesions, n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Acute†</td>
<td>Chronic†</td>
</tr>
<tr>
<td>Female</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PVG</td>
<td>c</td>
<td>68</td>
<td>20 (29)</td>
<td>1 (2)</td>
</tr>
<tr>
<td>PVG/WF</td>
<td>c/u</td>
<td>8</td>
<td>3 (38)</td>
<td>0</td>
</tr>
<tr>
<td>WAG</td>
<td>u</td>
<td>6</td>
<td>1 (17)</td>
<td>0</td>
</tr>
<tr>
<td>Male</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PVG</td>
<td>c</td>
<td>58</td>
<td>21 (36)</td>
<td>3 (5)</td>
</tr>
<tr>
<td>PVG/WF</td>
<td>c/u</td>
<td>24</td>
<td>12 (50)</td>
<td>5 (21)§</td>
</tr>
</tbody>
</table>

*No disease observed in Lou/m (n = 5), SHR (n = 6), DA (n = 5), or F344 (n = 4) strain female Tx-X rats maintained over the same period. †Hyperglycemia with insulin dependence. ‡Hyperglycemia without insulin dependence. §Itpatroph or insulitis in both diabetic and nondiabetic rats. $P < 0.05 compared with PVG female value. ¶P < 0.05 compared with PVG male value.
TABLE 6 Incidence of autoimmune lesions in lymphocyte-reconstituted or control Tx-X rats

<table>
<thead>
<tr>
<th>Lesion classification</th>
<th>Lymphocyte-reconstituted rats, n (%)</th>
<th>Control Tx-X rats, n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>25</td>
<td>47</td>
</tr>
<tr>
<td>Islet atrophy or insulin</td>
<td>2 (8)†</td>
<td>17 (36)‡</td>
</tr>
<tr>
<td>Thyroiditis</td>
<td>5 (20)§</td>
<td>28 (60)</td>
</tr>
</tbody>
</table>

*Each received 1 × 10⁸ viable syngeneic spleen cells intraperitoneally within 3 days of final irradiation. †P < 0.01 compared with control rats. §10 acute diabetic rats. §P < 0.001 compared with control rats.

The incidence of disease in males and females, whereas in NOD mice, a higher incidence of disease has been observed in females (4,23). Similarly, with respect to thyroiditis incidence in Tx-X PVG rats, a two- to fourfold increase in disease has been found in females, and in this situation, unequivocal evidence has been presented to show that steroid hormones exert a potent influence on thyroiditis expression (24,25). How these hormones act to produce differential effects on autoimmunity, both induction and protective, is uncertain and must await further clarification.

In earlier studies of autoimmune thyroiditis induced by the Tx-X procedure, we postulated that autoreactive cells occurred naturally in normal rats (14). The autoimmunity induced was attributable to a disturbance in equilibrium resulting from the selective depletion of a population of regulatory cells by the manipulation procedure (14,16). This hypothesis is equally relevant to IDDM development, and it would seem that both diabetes and thyroiditis have a common underlying pathogenesis. In this study, support for this hypothesis in relation to diabetes includes the demonstration of the presence of autoreactive cells by transfer from diabetic rats and also of regulatory cells by protection of Tx-X rats from diabetes by early reconstitution with normal lymphocytes. Experimental evidence supporting a regulator/effector cell imbalance also has been presented in other studies of diabetic rats. For example, McKeever et al. (21) reconstituted athymic nude rats with mitogen-activated, MHC-compatible, RT6-depleted lymphocytes from normal donors and induced both insulin and diabetes in a proportion of the recipients. Similarly, these workers were able to induce much higher incidence levels of diabetes when ConA-activated lymphocytes from acute diabetic rats were transferred to athymic MHC-compatible recipients. Fowell et al. (22) used the present model in a series of reconstitution studies in which fractionated T-cell populations derived from syngeneic normal donors were administered to Tx-X rats shortly after final irradiation. These studies showed also that diabetes can be suppressed by this means and provided clear evidence that the regulatory cell concerned is a helper T-cell of CD45 RC⁺ phenotype. Finally, evidence shows that the diabetes in BB rats can be prevented by the injection of RT6⁺ CD4⁺ peripheral T-cells from the congenic diabetes-resistant subline (26,27).

Data on diabetes onset after completion of irradiation indicated a window period of susceptibility. This short period may relate to a differential recovery of functional subtypes of lymphocytes after irradiation, leading to a temporary imbalance of autoreactive/regulatory cells. It would seem unlikely, however, that the postwindow decline in disease onset was caused by the recovery or regeneration of regulatory cells because we have shown that lymphocytes obtained from Tx-X rats at this stage are incapable of preventing thyroiditis onset when used to reconstitute other Tx-X rats (28).

Our data suggest that an imbalance between autoreactive and regulatory T-cell populations cannot be the sole factor determining the expression of autoimmunity. First, the data indicate a discordance between the onset of diabetes and thyroiditis. We have observed that the peak onset time of the two conditions does not coincide, both conditions are not always associated in the individual animal, and the overall incidence levels are different. It would be anticipated that if regulator/effector imbalance were the sole factor involved, both conditions would occur concurrently and with equal severity. Second, disease incidence never reaches 100% and is, moreover, strongly influenced by external environmental factors. This is exemplified in the current data of disease initiation in SPF versus conventionally derived rats in which striking differences in disease incidence levels were observed between the two groups. Similarly, in earlier studies, we found differences in incidence levels of thyroiditis in SPF compared with conventionally derived Tx-X groups (29). Because none of the recognized rat pathogenic viruses or bacteria were present in these animals, it was proposed that qualitative and quantitative differences in the microbial flora of the gastrointestinal tract, resulting from the hygienic standards under which the respective groups were derived, may have been the main environmental variable. In support of this concept we were able to manipulate the level of thyroiditis in SPF rats by the oral administration of gastrointestinal contents from non-SPF rats (29). Taken together, these data suggest that, in addition to the equilibrium disturbance induced by Tx-X procedure, environmental influences also are required to initiate the disease process.

In conclusion, the diabetic syndrome observed in Tx-X rats appears to closely parallel the human condition in many respects. Furthermore, the lack of a requirement for immunization makes it likely that the epitopes involved and the manner of their presentation are those implicated in the natural disease process. The suitability of this model for genetic studies, and the ability to manipulate it either towards disease induction or inhibition should provide further scope for detailed study of the pathogenesis of diabetes.

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