Thymectomy and Radiation-Induced Type 1 Diabetes in Nonlymphopenic BB Rats

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Spontaneous type 1 diabetes in BB rats is dependent on the RT1u MHC haplotype and homozygosity for an allele at the Lyp locus, which is responsible for a peripheral T-lymphopenia. Genetic studies have shown that there are other, as yet unidentified, genetic loci contributing to diabetes susceptibility in this strain. BB rats carrying wild-type Lyp alleles are not lymphopenic and are resistant to spontaneous diabetes (DR). Here we show that thymectomy and exposure to one sublethal dose of γ-irradiation (TX-R) at 4 weeks of age result in the rapid development of insulitis followed by diabetes in 100% of DR rats. Administration of CD4+ 45RC– T-cells from unmanipulated, syngeneic donors immediately after irradiation prevents the disease. Splenic T-cells from TX-R–induced diabetic animals adoptively transfer type 1 diabetes to T-deficient recipients. ACI, WF, WAG, BN, LEW, PVG, and PVG.RT1u strains are resistant to TX-R–induced insulitis/diabetes. Genetic analyses revealed linkage between regions on chromosomes 1, 3, 4, 6, 9, and 16, and TX-R–induced type 1 diabetes in a cohort of nonlymphopenic F2 (Wistar Furth × BBDP) animals. This novel model of TX-R–induced diabetes in nonlymphopenic BB rats can be used to identify environmental and cellular factors that are responsible for the initiation of antipancreatic autoimmunity. Diabetes 51:2975–2981, 2002

The BioBreeding (BBDP) rat spontaneously develops a T-cell–mediated, autoimmune diabetic syndrome that is similar to that observed in NOD mice and humans (1). Two of the diabetes susceptibility loci of the BB rat have been identified, Iddm1, which maps to the Lyp locus on chromosome 4, and Iddm2, which maps to the MHC class II haplotype RT1u of this animal (2,3). The Lyp allele carried by the BDP rat shortens the life span of naïve T-cells, resulting in a 5- to 10-fold decrease in the number of peripheral T-cells (4,5).

It remains unclear when and how this peripheral T-lymphopenia contributes to the development of diabetes, although its contribution is likely multifactorial.

Our understanding of the pathogenic role of the BBDP T-lymphopenia is further complicated by the demonstration that autoimmune diabetes can develop in nonlymphopenic BBDR rats, a strain that is genetically related to BBDP rats (6,7; www-genome.wi.mit.edu/rat/public/). BBDR rats are not lymphopenic and do not develop diabetes when maintained in a specific pathogen–free (SPF) environment (8). Although there is >15% genetic polymorphism between BBDP and BBDR rats (www-genome.wi.mit.edu/rat/public/), spontaneous diabetes cosegregates as a single gene with the T-lymphopenia between these two lines (9). Experimental induction of a peripheral T-lymphopenia in BBDR rats, through the administration of a depleting monoclonal antibody, cyclophosphamide, or sublethal γ-irradiation, is followed by the rapid development of diabetes in a conventional environment (10). However, diabetes can also occur in unmanipulated BBDR rats after infection with Kilham’s rat virus (KRV), a single-stranded DNA parvovirus with no tropism for β-cells, or injection of polyinosinic-polycytidylic acid (poly[I:C]), an interferon-α–inducing agent (11,12). Induction of diabetes by administration of poly(I:C) is observed both in SPF conditions and in conventional environment (12,13). Importantly, susceptibility to these experimentally induced type 1 diabetic syndromes is not restricted to BB-related strains as long as the animals are haploidentical to BB rats at the MHC class II locus (12,14). This observation suggests that diabetes susceptibility alleles are widespread among laboratory rats.

This interpretation is further supported by the demonstration that another type 1 diabetic syndrome can be induced in various strains of rats, including some that do not carry the RT1u MHC haplotype (15). Specifically, adult thymectomy followed by four subsequent, sublethal doses of γ-irradiation (TX-R) given 2 weeks apart results in the development of diabetes 10 weeks after irradiation. Strains susceptible to TX-R–induced diabetes include PVG (RT1u), PVG (RT1n), WAG (RT1n), and (PVG × WF)F1 (RT1n/c) (15,16). Therefore, it seems that most of the experimentally induced type 1 diabetic syndromes require the BB rat MHC class II haplotype, but none requires the BB rat genetic background exclusively. Here we describe a novel model of experimentally induced diabetes that is restricted to nonlymphopenic, BB-related strains.
RESEARCH DESIGN AND METHODS

Animals. Diabetes-resistant BBDR and diabetes-prone BBDP rats were purchased from BMR (Worcester, MA) and maintained in our colony under SPF and virus antibody-free (VAF) conditions. Specifically, all immunocompetent sentinels remained serologically negative for the KRV as well as for other viruses (SDAV, Sendai, PVM, Reo3, TMEV, GDVII, MADI, parvovirus, and LCMV) during the course of the study. Diabetes-prone BB.7b rats that are congenic for the RT1b allele of Wistar Furth (WF) rats into BBDR rats, followed by >10 backcrosses to BBDR rats (17). The cumulative incidence of diabetes in BB.7b animals is similar to that observed in BBDR rats originating from the BMR colony (data not shown). Nonlymphopenic and hence diabetes-resistant (nonly BB/W) rats have been generated by introgressing the wild-type Lyp allele from BBDE into BBDR rats, followed by systematic backcrossing to BBDP rats. Nonly BB/W animals used in this study were the progeny of the seventh backcross. Diabetes-prone and lymphopenic DR.Lyp/Lyp as well as diabetes-resistant and nonlymphopenic DR.Lyp+/− congenic lines were obtained from Dr. A. Lernmark (Washington University, Seattle, WA) and have been previously described (9). Other rat strains were purchased from Harlan Sprague-Dawley (Indianapolis, IN). ACI rats congenic for RT1u and ACI.Rt1u.lyp rats congenic for both RT1u and the Lyp allele of BBDP rats have been previously described (18). F1, F2, and F3 rats were generated in our animal colony.

Thymectomy, sham thymectomy, and whole-body irradiation of rats were performed as previously described (5). Rats were tested three times a week for the presence of glycosuria and ketonuria. Once the animals became hyperglycemic (blood glucose >16.7 mmol/l) for two consecutive days. Diabetic rats were treated with subcutaneous implants of insulin (Limplant; University of Toronto, ON, Canada). After the rats were killed, pancreas, lung, kidney, and liver were fixed in 10% formalin for histology. All of the animal protocols were approved by the Animal Care Committee of our institution.

Monoclonal antibodies, three-color immunofluorescence, and fluorescence-activated cell sorter analysis. The monoclonal antibodies (mAbs) used in this study have been previously described (5). Suspensions of MNC were incubated with a biotinylated mAb, followed by streptavidin-phycoerythrin (CFY/ Texas Red Tandem. PE- and FITC-conjugated mAbs were then added simultaneously. Cells were then analyzed by flow cytometry on a Becton Dickinson (San Jose, CA) FACScalibur or sorted using a MoFlo (Cytomation, Denver, CO). At least 104 cells/sample were acquired for analysis.

Isolation of T-cell subpopulations. T-cells were enriched by negative selection using a rosetting procedure as previously described (19,20). The purity of T-cells obtained from nonlymphopenic animals by rosetting was routinely >98%. Furthermore, different subsets of CD4+ T-cells and T-depleted splenocytes were purified by fluorescence-activated cell sorting (FACS).

Genetic analyses. The T-lymphopenic status of F1(WF × BBDP) animals was determined on peripheral blood by immunofluorescence and FACS analysis. Genomic DNA was prepared from tail snips using standard methods. Nonlymphopenic F2 animals (n = 102) were followed for type 1 diabetes and insulinus induced by TX-R. The animals were killed when they developed type 1 diabetes or 2 months after irradiation. After the rats were killed, the pancreata was fixed in 10% formalin for histological analysis. All microsatellite primers used in this study were obtained from Genosys, Sigma (Cambridge, U.K.). The genetic map location of the markers was obtained from the Whitehead Institute/MIT Center for Genome Research web site (www.genome.wi.mit.edu/rat/public). Primers were tested against parental DNA samples to confirm the polymorphism between BBDP and WF rats. Polymorphic primers were then used in a genome-wide screen. Information on the primers used and tested is available at www.well.ox.ac.uk/rat_mapping_resources/. The genome scan was performed at ~15- to 20-cM interval. Genotyping was performed by PCR amplification on 50 ng of genomic DNA. PCR products were separated by electrophoresis on standard denaturing sequencing gels and transferred onto nylon membranes. The membranes were hybridized with a probe labeled with (α-32P) dCTP using terminal transferase (21). Genotypes were independently scored by two observers (M.-T.B. and S.R.). Crosses were validated by verifying the linkage of lymphopenia with the appropriate markers on chromosome 4 (2).

Statistical analyses. A genetic map was built using MAPMAKER EXP based on the order of polymorphisms on the rat genome provided at www.genome.wi.mit.edu/rat/public. Quantitative trait linkage analysis was performed using the MAPMAKER EXP v3.0 and QTLa-Link programs (www-genome.wi.mit.edu/genome_software/). The marker data were analyzed for linkage to each of the traits studied using MAPMAKER QTL, with the genetic model with the highest LOD score chosen as the best model. The appropriate LOD score, proportion of the trait variance explained at the locus, and the markers that showed LOD scores >1.5 were noted. Probabilities were independently confirmed using ANOVA. Basically, it is an ANOVA test followed by permutation tests that provide exact significance thresholds for each pair of genotypic/diabetic phenotype, regardless of the genotype distribution in the cross. This method is therefore particularly appropriate for nonparametric analyses was developed by Churchill and Doerge (22) and was used recently by Martin et al. (23) in their QTL analyses. We have conformed to the guidelines proposed by Landen and Kruglyak (24) in interpreting the statistical significance of the findings.

RESULTS

Thymectomy and sublethal irradiation induce diabetes in BBDR rats in an age-dependent manner. In the course of adoptive transfer experiments using bone marrow radiation chimera, we observed that BBDR rats exposed to TX-R rapidly developed diabetes with a high incidence. We decided to further characterize this experimentally induced diabetic syndrome.

When 4-week-old BBDR rats were thymectomized and, 1 week later, received one sublethal dose of 5 Gy of TX-R, 100% of the animals (31 of 31) developed diabetes 21–35 days after irradiation with a mean onset of 28 ± 6 days (Table 1). Both sexes were susceptible. The diabetic syndrome was characterized by the acute development of polyuria, polydipsia, weight loss, hyperglycemia, glycosuria, and ketonuria. Diabetic animals required daily insulin injections to survive. Prospective, histological analysis of the pancreas performed weekly after irradiation showed that diabetes was preceded by the development of insulin (Fig. 1). Infiltration of pancreatic islets by MNCs was a late and rapid event that became detectable only a few days before and disappeared rapidly after the onset of diabetes, leaving in place end-stage islets with no insulin-containing cells (data not shown). No inflammation was observed in the exocrine pancreas, lungs, kidneys, and liver of thymectomized and irradiated rats.

As illustrated in Table 2, susceptibility to TX-R-induced diabetes was age dependent. Specifically, thymectomy had to be performed between 3 and 5 weeks, and sublethal irradiation had to be performed within 1 week after

<table>
<thead>
<tr>
<th>Strain</th>
<th>MHC haplotype</th>
<th>Type 1 diabetes</th>
<th>Insulitis</th>
<th>Mean day of onset after R</th>
</tr>
</thead>
<tbody>
<tr>
<td>BBDR</td>
<td>RT1u/a/a</td>
<td>31/31</td>
<td>NA</td>
<td>28 ± 6</td>
</tr>
<tr>
<td>DR.Lyp/+</td>
<td>RT1u/a/a</td>
<td>6/6</td>
<td>NA</td>
<td>34 ± 5</td>
</tr>
<tr>
<td>Non-lyp BB/W</td>
<td>RT1u/a/a</td>
<td>7/7</td>
<td>NA</td>
<td>31 ± 3</td>
</tr>
<tr>
<td>ACI</td>
<td>RT1u/a/a</td>
<td>0/5</td>
<td>0/5</td>
<td></td>
</tr>
<tr>
<td></td>
<td>RT1u/a/a</td>
<td>0/7</td>
<td>0/7</td>
<td></td>
</tr>
<tr>
<td>ACI</td>
<td>RT1u/a/a</td>
<td>0/5</td>
<td>0/5</td>
<td></td>
</tr>
<tr>
<td>BN</td>
<td>RT1v/v</td>
<td>0/5</td>
<td>0/5</td>
<td></td>
</tr>
<tr>
<td>LEW</td>
<td>RT1f/f</td>
<td>0/4</td>
<td>0/4</td>
<td></td>
</tr>
<tr>
<td>PVP</td>
<td>RT1c/c</td>
<td>0/4</td>
<td>0/4</td>
<td></td>
</tr>
<tr>
<td>WAG</td>
<td>RT1u/a/a</td>
<td>1/6</td>
<td>1/5</td>
<td>47</td>
</tr>
<tr>
<td>WF</td>
<td>RT1u/a/a</td>
<td>0/9</td>
<td>0/9</td>
<td></td>
</tr>
<tr>
<td>(WF × BBDP)</td>
<td>RT1u/a</td>
<td>7/8</td>
<td>1/1</td>
<td>48 ± 8</td>
</tr>
<tr>
<td>(BBDP × WF)</td>
<td>RT1u/a</td>
<td>4/4</td>
<td>NA</td>
<td>45 ± 4</td>
</tr>
<tr>
<td>(WF × BBDR)</td>
<td>RT1u/a</td>
<td>6/16</td>
<td>5/10</td>
<td>37 ± 1</td>
</tr>
<tr>
<td>(WF × DR.Lyp/)</td>
<td>RT1u/a</td>
<td>8/11</td>
<td>0/3</td>
<td>35 ± 5</td>
</tr>
<tr>
<td>(ACI.1u × BBDR)</td>
<td>RT1u/a</td>
<td>0/4</td>
<td>0/4</td>
<td></td>
</tr>
<tr>
<td>(ACI.1u × BBDR)</td>
<td>RT1u/a</td>
<td>0/5</td>
<td>0/5</td>
<td></td>
</tr>
</tbody>
</table>

*Insulitis in nondiabetic animals. NA, not applicable.
thymectomy. Exposure to TX-R or thymectomy alone consistently failed to induce diabetes.

**TX-R–induced diabetes is a T-cell–mediated autoimmune disease.** The presence of insulitis before the development of the disease strongly suggested that TX-R–induced diabetes was autoimmune in nature. To determine whether this is the case, we performed an adoptive transfer of MNC from acutely diabetic TX-R rats to nondiabetic recipients. We used recipients that were both MHC identical to the donors and T-cell–deficient, WAG rats homozygous for the nude allele, and 4-week-old, sublethally irradiated BB-7b rats. Adoptively transferred populations of lymphocytes (2 × 10^6 cells intravenously) included sorted, CD3^- splenocytes and splenocytes enriched (70–80%) in T-cells by rosetting. All of the recipients of splenic T-cells developed diabetes within 4 weeks after transfer (Table 3), whereas none of the animals that received CD3^- splenocytes or were left untreated did. No insulitis was found in the recipients that had not become diabetic and were killed 8 weeks after transfer (data not shown). These results demonstrate that TX-R–induced diabetes is a T-cell–mediated autoimmune disease.

**Syngeneic CD45RC\(^{-}\), CD4\(^{+}\) T-cells prevent induction of TX-R–induced diabetes.** As expected, TX-R resulted in peripheral T-lymphopenia in BBDR rats. Specifically, T-cells accounted for 6 ± 1.3% and 22 ± 4% (n = 7) of splenic and lymph node MNCs, respectively (Fig. 2), at the onset of diabetes. Both spontaneous diabetes in BBDR rats and the diabetic syndrome induced in PVG.RT1\(^{a}\) rats by adult thymectomy and multiple, low doses of TX-R are also associated with T-lymphopenia, and, in both cases, it has been demonstrated that the lymphopenia plays a central role in disease pathogenesis (25,26). Furthermore, it has been shown that a lack of regulatory T-cells is one of the pathogenic mechanisms of the T-lymphopenia in both BBDR and PVG.RT1\(^{a}\) rats (25,26). Specifically, reconstitution of prediabetic rats with RT6\(^{a}\), CD4\(^{+}\) T-cells, in the case of BBDR rats, and CD45RC\(^{-}\), CD4\(^{+}\) T-cells, in the case of PVG.RT1\(^{a}\) animals, prevented diabetes (25,26).

These observations led us to assess the ability of various T-cell subsets to modulate TX-R–induced diabetes in BBDR rats. TX-R rats received unfractionated T-cells or purified T-cell subsets isolated from adult, unmanipulated BBDR donors immediately after irradiation. The T-cell subsets consisted of CD4\(^{+}\), CD8\(^{+}\), CD45RC\(^{-}\), CD4\(^{+}\), or CD45RC\(^{-}\) CD4\(^{+}\) T-cells. As illustrated in Table 4, as few as 2 × 10^6 unfractionated T-cells, CD4\(^{+}\) T-cells, and CD45RC\(^{-}\) CD4\(^{+}\) T-cells afforded protection from diabetes in 100% of the recipients. In contrast, reconstitution of TX-R BBDR rats with up to 5 × 10^6 CD45RC\(^{+}\) CD4\(^{+}\) T-cells or up to 2 × 10^6 CD8\(^{+}\) T-cells was not protective. Importantly, the ability of unfractionated T-cells to prevent diabetes was lost when T-cell reconstitution was delayed by 1 and 2 weeks, suggesting that the autoimmune process is initiated soon after irradiation and/or expansion of regulatory T-cells is required before the initiation of the diabetogenic process. These results demonstrate that the diabetic syndrome induced by TX-R in BBDR rats is amenable to T-cell regulation.

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**TABLE 2**

<table>
<thead>
<tr>
<th>Age (weeks)</th>
<th>Type 1 diabetes</th>
<th>Insulitis*</th>
</tr>
</thead>
<tbody>
<tr>
<td>TX 5 Gy</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>5</td>
<td>31/31</td>
</tr>
<tr>
<td>4</td>
<td>8</td>
<td>0/5/0/5</td>
</tr>
<tr>
<td>4</td>
<td>6</td>
<td>0/5/0/5</td>
</tr>
<tr>
<td>6</td>
<td>8</td>
<td>0/5/0/5</td>
</tr>
<tr>
<td>8</td>
<td>9</td>
<td>0/4/0/4</td>
</tr>
<tr>
<td>4</td>
<td>—</td>
<td>0/4/0/4</td>
</tr>
<tr>
<td>—</td>
<td>5</td>
<td>0/5/0/5</td>
</tr>
</tbody>
</table>

*Insulitis in nondiabetic animals.

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**TABLE 3**

Adoptive transfer of type 1 diabetes by T-cells from diabetic TX-R, RT7\(^{a}\), BBDR rats

<table>
<thead>
<tr>
<th>Donor cells</th>
<th>Recipient</th>
<th>IDDM</th>
<th>Insulitis*</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>5Gy BB-7b</td>
<td>0/5</td>
<td>0/5</td>
</tr>
<tr>
<td>Splenic T-cells</td>
<td>5Gy BB-7b</td>
<td>4/4</td>
<td>NA</td>
</tr>
<tr>
<td>WAG rnu/rnu</td>
<td>WAG rnu/rnu</td>
<td>4/4</td>
<td>NA</td>
</tr>
<tr>
<td>T-depleted splenocytes</td>
<td>5Gy BB-7b</td>
<td>0/4</td>
<td>0/4</td>
</tr>
</tbody>
</table>

T-cells were enriched by rosetting and were injected intravenously into WAG rnu/rnu rats or 4-week-old, sublethally irradiated BB7b rats. *Insulitis in nondiabetic animals.
TX-R-induced diabetes is observed only in BBDP-related strains. We next examined whether susceptibility to TX-R-induced diabetes is genetically determined by exposing nonlymphopenic, BBDP-related strains as well as other BB-unrelated strains to TX-R. Several inbred strains of nonlymphopenic, RT1u rats, including BBDR animals, were derived from BBDP animals at various times and for different purposes. These strains exhibit ~15% of genetic polymorphism across the whole genome with BBDP rats (www.genome.wi.mit.edu/rat/public). The two BBDP-related strains that were studied were nonlyp BB/W and DR,Lyp/+ rats (9). Two other groups of strains unrelated to BBDP rats were tested for susceptibility to TX-R-induced diabetes. One group, comprised of WF, WAG, PVR.T1u, and ACI.Lyp rats carry the same MHC RT1u haplotype as BBDP and BBDR rats. The other group was composed of animals carrying non–u RT1 haplotypes, namely LEW (RT1v), BN (RT1v), ACI (RT1v), and PVR (RT1v) rats. All of the rats were followed for up to 3 months after TX-R.

After TX-R, 100% of nonlymphopenic, BBDP-related animals developed diabetes with comparable kinetics (Table 1). In contrast, none of the BBDP-unrelated animals became diabetic, except for one PVR.T1u rat (one of six). None of the nondiabetic rats had insulitis at the time they were killed (data not shown). These results demonstrate that nonlymphopenic animals that are genetically related to the BBDP strain are uniquely susceptible to TX-R-induced diabetes. At this stage and in the absence of nonlymphopenic, BBDP-related rats congenic for a non–u RT1 haplotype, the role of MHC genes in susceptibility to TX-R-induced diabetes remains unknown.

Genetic linkage analysis of TX-R-induced diabetes. We decided to characterize further the genetic basis of the susceptibility of nonlymphopenic, BBDP-related rats to TX-R-induced diabetes. In a first step, we determined the incidence of diabetes in F1 animals resulting from a cross between BBDP-related and either WF- or RT1-congenic ACI rats (Table 1). Diabetes was observed in a large proportion of F1(WF × BBDP-related) and F1(BBDP-related × WF) animals, demonstrating that in these crosses, TX-R–induced diabetes is inherited as a dominant trait with variable penetrance. However, none of the nine F1(BBDR × congenic ACI) animals became diabetic. Although the number of these F1(BBDR × congenic ACI) is low, the lack of diabetics among them is consistent with the interpretation that the ACI background carries factors of resistance to TX-R-induced diabetes. Importantly, we have previously provided evidence for the presence of factors of resistance to spontaneous diabetes in the ACI genetic background (18).

In a second step, we performed a segregation analysis of diabetes and insulitis in a cohort of 102 nonlymphopenic F2(WF × BBDP) rats. We selected these parental strains for several reasons. Both strains carry the RT1u haplotype. The reported degree of genetic polymorphism between WF and BBDP rats, ~35%, is relatively high (www.genome.wi.mit.edu/rat/public). The highest incidence of TX-R–induced diabetes among F1(WF × BBDP-related) animals was observed in the progeny of WF × BBDP crosses. Animals were exposed to TX-R at 4 weeks and followed prospectively for diabetes up to 10 weeks after irradiation. Inheritance of the T-lymphopenia showed Mendelian segregation because 25.2% of the F2 animals were lymphopenic.

A genome-wide scan of F2 animals was performed using 117 markers polymorphic between BBDP and WF. Regions on chromosome 5 (between D5rat108 and D5rat50, ~30 cM), chromosome 10 (top to D10rat85, ~40 cM), and chromosome 14 (top to D14rat24, ~28 cM) were not analyzed because of the lack of polymorphic markers distinguishing the two parental strains at these sites. Additional markers were tested in areas that showed some evidence for linkage.

Diabetes and insulitis developed in 43.1 and 81% of the animals, respectively. Both sexes were equally represented in each of the phenotypic categories (data not shown). All of the animals were BB/WF or WF/WF in the Lyp region on chromosome 4 (D4got54) because lymphopenic animals were excluded from the study. The microsatellite markers that showed significant linkage to diabetes and/or insulitis with LOD scores >1.5 are shown in Table 5. Linkages to diabetes and insulitis were observed on chromosomes 1, 3, 4, 6, 9, and 16.

DISCUSSION
This study describes a novel, autoimmune, type 1 diabetic syndrome that can be rapidly induced in BBDP-related
strains with a very high incidence. Many features of this diabetic syndrome distinguish it from those induced in RT1<sup>i</sup> strains through viral infection, administration of poly I:C alone or in combination with anti-RT6 antibody, or in PVG rats by thymectomy and multiple low doses of TX-R (15,26). Susceptibility to diabetes induced by KRV and poly I:C is widely distributed in nonlymphopenic, RT1<sup>i</sup>-expressing strains, whereas TX-R–induced diabetes seems to be restricted to BB-related strains (11,12). The latter syndrome may therefore prove helpful in identifying diabetes susceptibility factors that are peculiar to the BB genetic background and possibly contribute to both spontaneous and experimentally induced diseases.

The diabetic syndrome induced by thymectomy and multiple low doses of TX-R occurs in PVG animals, independent of their RT1 haplotype, but also in WAG rats, two strains shown here to be resistant to TX-R–induced diabetes (Table 1). Adoptive transfer of diabetes induced by thymectomy and multiple low doses of TX-R to irradiated, syngeneic recipients by splenic T-cells was unsuccessful despite preactivation of donor T-cells by ConA in vitro (16). Furthermore, only a small proportion of the T-cell recipients developed lesions of insulitis. In contrast, all recipients of splenic T-cells freshly isolated from TX-R–induced diabetic donors developed diabetes in a few weeks. The time constraints for successful induction of diabetes through thymectomy and multiple low doses of TX-R seem less stringent than those required for TX-R–induced diabetes. Specifically, induction of the former diabetic syndrome requires that thymectomy be performed between 3 and 6 weeks and irradiation be initiated 2 weeks later, whereas in the case of the latter diabetic syndrome, thymectomy has to be performed in animals that are ≤4 weeks old and irradiation cannot be postponed beyond 1 week after thymectomy (Table 2). It remains, however, that in both diabetic syndromes, the experimental procedure seems to affect the development and/or function of regulatory T-cells with the CD45RC<sup>+</sup> CD4<sup>+</sup> TeRo<sup>+</sup> membrane phenotype because reconstitution of this subset immediately after irradiation prevents the development of the disease (26) (Table 4).

It has been previously reported that type 1 diabetes can be induced in BBDR rats by a single sublethal dose of TX-R (10). In our hands, this protocol remained unsuccessful, independent of the dose of irradiation and age of the animals (Table 2 and data not shown). We believe that this discrepancy is related to environmental factors. The Worcester colony of BBDR rats, the source of our animals, became VAF through cesarean rederivation in 1990. Before that transfer, the animals were kept in SPF conditions (27). Changes in the susceptibility of BBDR rats to experimentally induced diabetic syndromes were associated with the breeding of these animals in VAF conditions. For example, treatment of BBDR rats with a depleting mAb specific for RT6, an ADP-ribosyltransferase expressed on the surface of most mature rat T-cells, was diabetogenic in SPF conditions but not in a VAF environment (27). Similarly, induction of diabetes in BBDR rats by a single sublethal dose of TX-R was possible at a time when these animals were maintained in SPF conditions but remained unsuccessful in our VAF facility (10) (Table 2). It remains unclear at this stage how thymectomy can be substituted for exposure to SPF conditions. However, it has been shown in models of autoimmune thyroiditis and diabetes induced by thymectomy and multiple low doses of TX-R that the thymus contains regulatory T-cells that prevent autoimmunity (28). Furthermore, in the case of autoimmune thyroiditis, the development of these regulatory T-cells required the presence of a functional thyroid gland (29).

One of the intriguing aspects of spontaneous and experimentally induced diabetes in BBDR-related strains is that the various manipulations that prevent or precipitate diabetes have to be performed before 4 weeks (30,31). Specifically, reconstitution of diabetes-prone BBDP rats with normal T-cells protects the recipients from diabetes, provided that the protective T-cells are injected in the first 4 weeks of life (30). Thymectomy of BBDR rats prevents diabetes when performed in ≤4-week-old animals but does not affect the time course and incidence of the disease when delayed beyond that age (31; S. Ramanathan and P. Poussier, unpublished observations). There is evidence in the NOD mouse that activation of diabetogenic T-cells by their specific β-cell antigens occurs in pancreatic lymph nodes around the age of 2 weeks (32). Whether this early and potentially deleterious presentation of islet cell antigens is a physiological phenomenon remains an open question. The present study and others have demonstrated that potentially diabetogenic T-cells are present in the pool of recirculating T-cells of unmanipulated BBDR rats (17,26). Presentation of self-antigens to their specific T-cells, if it occurs in unmanipulated BBDR rats, must result in T-cell tolerance or ignorance because these animals remain diabetes free. Here we provide evidence, although indirect, that either presentation of islet cell antigens is restricted to the first 4 weeks of life or, in the event that this self-presentation persists beyond 4 weeks, it can no longer prime a deleterious autoimmune response in BBDR-related strains.

Assuming that potentially deleterious presentation of β-cell antigens persists throughout the life of BBDR rats, our results suggest that thymectomy followed by TX-R has a differential effect on the homeostasis and/or repertoire of peripheral T-cells in young and adult animals. CD45RC<sup>−</sup>CD4<sup>+</sup> TeRo<sup>+</sup> T-cells shown here and in another model of diabetes to prevent autoimmunity account for a low proportion of peripheral T-cells in young animals (33). The proportion of this regulatory T-cell subset increases as the contribution of recent thymic emigrants to the pool of recirculating T-cells decreases with age. It is not implausible that the differential effect of thymectomy and TX-R on diabetes susceptibility in young and adult animals is related to age-related changes in the repertoire of peripheral T-cells. However, the peripheral T-lymphopenia is so severe immediately after irradiation that we could not detect reliable differences in the proportions of naïve and memory T-cells between young and adult rats.

Genetic analysis of TX-R–induced diabetes showed weak linkage of the disease to regions on Ch 1, 3, 4, 6, 9, and 16 (Table 5). This absence of robust linkages suggests that the development of insulitis and clinical disease is under the regulation of multiple genes and that most of these non-MHC loci make only incremental contribution to the diabetogenic process. Our analysis relied on complex
phenotypes, diabetes and insulitis, that most likely result from the action of many genes involved in complex pathways, and, furthermore, it was performed in a limited number of F2 animals. It is therefore not surprising that the influence of individual loci proved difficult to assess. Similar difficulties in genome-wide mapping of diabetes susceptibility loci have also been observed in other human and murine diabetic syndromes for similar reasons (34–36). One way to overcome these difficulties is to identify preclinical, disease-related phenotypes and determine the genetic loci that govern them. This approach has helped in identifying Idd loci that control various stages of insulitis and progression to diabetes in the NOD model (37). An additional factor that complicates the search for diabetes-susceptibility loci in rats is that several inbred strains of RT1u+ animals seem to carry susceptibility alleles to experimentally induced diabetes (12). Outside the MHC class II and Lyp loci, 4–6 Iddm loci have been linked to spontaneous diabetes (38–41) and up to three to experimentally induced diabetic syndromes (23,42). In our study, we did not observe linkage to any of these previously reported Iddm loci, which suggests that variants at non-MHC loci conferring risk to type 1 diabetic syndromes in rats are not all identical. It is possible, however, that many of these genes function in conserved pathways central to diabetes pathophysiology.

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REFERENCES


