Elevated mRNA Levels of Major Histocompatibility Complex Class II Genes in Lymphocytes of Autoimmune BB Rats

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The BB rat spontaneously develops autoimmune abnormalities such as insulin-dependent diabetes mellitus and thyroiditis. The autoimmunity of the BB rat is controlled in part by genes of the major histocompatibility complex (MHC), known as the RT1 complex in the rat, and accumulating evidence suggests the involvement of MHC class II molecules. The RT1 complex specifies two types of class II molecules, which are encoded by the loci RT1.B and RT1.D. We have determined the relative steady-state mRNA levels of the class II genes RT1.Bβ, RT1.Dα, and RT1.Dβ in splenic lymphocytes from individual autoimmune BB rats of various ages and from age-matched histocompatible normal Wistar-Furth (WF) rats. The relative steady-state mRNA levels of the RT1.Dα and RT1.Dβ genes, but not of the RT1.Bβ gene, were elevated ~2.5-fold in lymphocytes of prediabetic BB rats 45–75 days old in comparison with age-matched normal WF rats and older BB rats >75 days old. In the diabetic and nondiabetic BB rats >75 days old, the RT1.Dα and RT1.Dβ transcripts were found at lower normal levels, similar to that of WF rats. In contrast, the RT1.Bβ transcripts were found at comparable levels in lymphocytes of the BB and WF rats at all ages examined. The increased steady-state mRNA levels of the RT1.Dα and RT1.Dβ genes in the prediabetic BB rats may reflect differences in the proportion of lymphocytes expressing these genes and thus differences in splenic lymphocyte populations. These elevated steady-state mRNA levels of both RT1.D genes in lymphocytes of prediabetic BB rats correlate with the age when the autoimmune effector mechanisms are highly activated and suggest an involvement of RT1.D class II antigens with the autoimmune disease process in the BB rat. The differences in steady-state class II mRNA levels in prediabetic BB rats and the age-matched normal WF rats and older BB rats suggest that the RT1.Dα and RT1.Dβ genes are similarly regulated, but not coordinately regulated with the RT1.Bβ gene, in splenic lymphocytes of young autoimmune BB rats.
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The Wistar-derived BB rat spontaneously develops autoimmune abnormalities such as insulin-dependent diabetes mellitus (IDDM) and thyroiditis (1). The development of IDDM in BB rats, as in humans, is characterized by insulitis followed by the specific and selective elimination of the pancreatic β-cells (2). The incidence of IDDM development in autoimmune inbred BB rats is ~40–60%. Susceptibility to the development of IDDM is controlled in part by genes of the major histocompatibility complex (MHC), known as the RT1 complex in the rat. Breeding studies have demonstrated the requirement of the $RT1^u$ haplotype and have implicated the RT1-complex class II molecules in the autoimmune response of the BB rat (3–5).

The RT1 complex specifies two types of class II molecules encoded by the loci RT1.B and RT1.D, which are analogous with the *H-2* I-A and I-E loci of the mouse, respectively, and with the human loci HLA-DQ and HLA-DR, respectively (6). The class II molecules function in regulating and guiding immune responses in the rat (6) as well as in the mouse and human (7,8). It has generally been assumed that the close association between IDDM and specific alleles of MHC class II genes was due to the regulatory role of the class II molecules in the immune response. Support for this hypothesis was found in the immunosuppression of IDDM and thyroiditis development in the BB rat with the in vivo administration of locus-specific anti-RT1.D monoclonal antibodies (9).

In our studies on the possible role of the class II molecules in susceptibility to the development of autoimmune disease in the BB rat, we isolated and characterized class II cDNA genes of the BB rat (10,11; E.W.H. and M.K.G., unpublished observations). We have previously shown that the steady-

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state level of RT1.Dα mRNA in splenic lymphocytes of young autoimmune BB rats is elevated (11). In this article, we have extended these studies to include the steady-state mRNA levels of class II genes from both loci, RT1.B and RT1.D, in splenic lymphocytes of autoimmune BB rats and age-matched histocompatible normal Wistar-Furth (WF) rats.

MATERIALS AND METHODS

Animals. The BB and WF rat strains express the serotypically defined $RT1^u$ haplotype. Diabetes-prone autoimmune BB rats were obtained from the Toronto colony (Hospital for Sick Children) or the Ottawa colony (P. Thibert), and hooded BB rats were obtained from J. Logothetopoulos (University of Toronto). Individual rats were identified as being diabetic on the basis of positive glycosuria, determined with Diastix and Ketostix reagent slips (Ames, Miles, Rexdale, Ontario, Canada). The average age at IDDM development in the BB rats was 95 days, the youngest being 78 days. BB rats were killed within 10 days of diabetes detection, and those that did not develop diabetes were killed by 120 days of age. The normal WF rats were purchased from Harlan-Sprague-Dawley (Indianapolis, IN).

Purification of RNA. Spleen cells were isolated by piercing the splenic capsule with a 23-gauge needle at multiple sites and flushing repeatedly with RPMI-1640 medium. Approximately $2–6 \times 10^8$ splenocytes were recovered from individual rats >40 days old. Total cellular RNA was extracted from the washed splenic lymphocytes into guanidine isothiocyanate and purified by centrifugation through cesium chloride (12). The RNA samples were adjusted to 50 A_{260} units/ml, and RNA integrity was analyzed by electrophoresis on formaldehyde-agarose gels (13). $Poly(A)^+$ RNA was purified by two rounds of affinity chromatography on oligo dT-cellulose (14).

Analysis of MHC class II mRNA levels. Specific riboprobe plasmids were constructed for the RT1.Dα, RT1.Dβ, and RT1.Bβ genes as follows. These riboprobe plasmids carry the insert-template DNA in antisense orientation with respect to transcription initiation from the 17 RNA polymerase promoter. The RT1.Dα riboprobe pT7/RT1.$D\alpha^u$492 contains a 492–base-pair (bp) *Pst*I-*Sac*I DNA insert, which specifies amino acids 85–230 of the RT1.Dα molecule plus 49 bp of the 3′-untranslated region (10,11). The primary in vitro antisense transcript of 1166 ribonucleotides (nts) was made with *Sca*I-linearized template DNA, and 470 nt was homologous with RT1.Dα mRNA. The RT1.Dβ riboprobe plasmid was constructed by ligation of a 1589-bp *Bgl*II-*Eco*RI DNA fragment purified from a cDNA isolate of RT1.$D\beta^u$ (unpublished observation) into *Bam*HI-*Eco*RI–cleaved vector pT7/19. Of the 1589-bp fragment, 704 bp specified RT1.Dβ mRNA and encoded the amino acids 51–238 of the RT1.Dβ molecule plus 137 nt of the 3′-untranslated region, and 885 bp specified plasmid pBR322 DNA sequences. Primary antisense transcripts of 1612 nt were prepared with *Eco*RI–cleaved template DNA. The RT1.Bβ riboprobe plasmid was constructed by the blunt-end ligation of a 1069-bp *Stu*I-*Dra*I DNA fragment purified from a cDNA isolate of RT1.$B\beta^u$ (unpublished observation) into *Sma*I-cleaved pT7/1. The 1069-bp fragment included a 682-bp portion of RT1.Bβ cDNA, which specified amino acids 25–238 plus 41 nt of the 3′-untranslated region, and 387 bp of plasmid pBR322 DNA. The primary antisense transcript of 1133 nt was prepared with *Hind*III–cleaved template DNA.

Radioactively labeled antisense RNA was transcribed in vitro with T7 RNA polymerase, purified, hybridized with the target RNA at 50°C for 16 h, and processed with RNases A and T1, as previously described (11,15). Each ribonuclease protection assay was carried out with 10 μg (0.25 A_{260} units) of splenic lymphocyte RNA. The radioactive RNA fragments protected from RNase digestion by hybridization with complementary mRNA were resolved on 8 M urea, 5% polyacrylamide gels and displayed by autoradiography. Quantitation of protected RNA was performed by densitometry analysis of the autoradiograms with a Hoefer GS300 scanning densitometer and integration of the appropriate peak. To compare the results from different experiments, a standardized $poly(A)^+$ RNA sample was included with each set of experiments, which allowed normalization of the results. The use of total cellular RNA as target was preferred, in place of $poly(A)^+$ RNA, for comparisons of the relative levels of class II gene transcripts found in splenic lymphocytes of individual rats to circumvent the problems associated with the variable degree of purity found between different $poly(A)^+$ RNA purifications. The detection of class II mRNA was directly proportional to the amount of input target RNA within the tested range of 0.05–10.0 μg $poly(A)^+$ RNA or 0.5–20.0 μg total RNA (11).

RESULTS

The relative steady-state levels of transcripts specifying the MHC class II genes RT1.Bβ, RT1.Dα, and RT1.Dβ were measured in total cellular RNA purified from splenic lymphocytes of individual autoimmune BB rats and age-matched histocompatible normal WF rats. Our previous studies indicated that the relative levels of specific class II gene transcripts can be quantitatively assessed from total cellular RNA of splenic lymphocytes of individual rats with a RNase protection assay with radioactive antisense class II RNA probes (11). We compared the steady-state mRNA levels of specific class II genes in splenocyte cellular RNA of individual prediabetic, diabetic, and nondiabetic BB rats with those of normal nondiabetic histocompatible WF rats. The BB rats were placed in prediabetic group 1 (45–75 days old) or group 2 (78–120 days old), which included the BB rats that developed frank overt diabetes and nondiabetic BB rats 120 days old.

RNase-protection experiments were carried out by the hybridization in solution of total RNA samples with highly radioactive antisense class II RNA probes prepared from the RT1.Dα, RT1.Dβ, and RT1.Bβ riboprobe constructs. A summary of the RNase-protection studies is presented in Fig. 1. In the prediabetic group 1 BB rats, the relative steady-state mRNA levels of the RT1.Dα and RT1.Dβ genes were found at elevated levels of up to 2.5× greater when compared with the age-matched normal WF rats or with the group 2 BB rats. In contrast, the relative steady-state mRNA levels of the RT1.Bβ gene were found at similar levels in lymphocytes of both the BB and WF group 1 rats. The relative steady-state levels of mRNA specifying the RT1.D genes in lymphocytes of the diabetic and nondiabetic group 2 BB rats were found at levels similar to those of the WF rats. No significant differences in the relative levels of transcripts of

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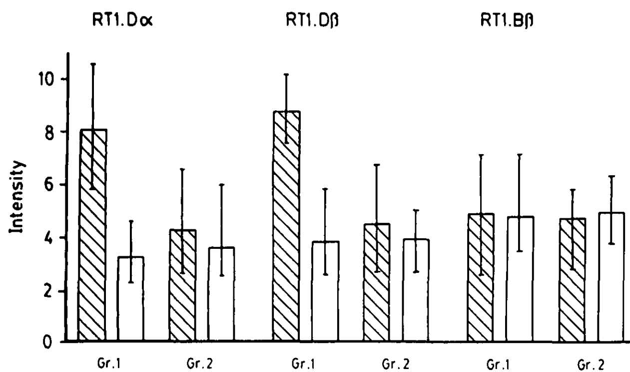

FIG. 1. Relative steady-state mRNA levels of major histocompatibility complex (MHC) class II genes detected in splenic lymphocyte RNA of autoimmune BB rats and age-matched normal Wistar-Furth (WF) rats. Relative mRNA levels were quantified by scanning densitometry of RNase-protection autoradiograms. Intensity of each RNA signal was comparable with others after normalization of signals on each autoradiogram with intensity found for standardized samples of 1 and 2 μg poly(A)$^+$ RNA. MHC class II gene specificity is indicated above each set of *bars*. *Bars* show average intensity for samples of group, and *vertical lines* indicate range of intensity. Total cellular RNA samples were prepared from BB rats (*hatched bars*) and age-matched normal WF rats (*open bars*) and included minimum of 10 rats each. Samples were grouped on basis of IDDM development and age: group 1 included prediabetic BB rats 45–75 days old, and group 2 included diabetic and nondiabetic BB rats 78–120 days old. Differences between steady-state mRNA levels of group 1 BB and group 1 WF, group 2 BB, and group 2 WF rats for class II genes RT1.Dα and RT1.Dβ were significant ($P < .001$).

the RT1.D genes in the group 2 rats were found between either the diabetic and nondiabetic BB rats (not shown) or between the BB rats and the age-matched WF rats. The steady-state mRNA levels of the RT1.Bβ gene in lymphocytes of the group 2 rats were also found at similar levels in BB and WF rats. The average relative level of transcripts specifying the RT1.Bβ gene did not vary significantly between the BB and WF rats regardless of age. These patterns of class II gene steady-state mRNA levels were observed in splenic lymphocytes of BB rats originating from three independent colonies.

DISCUSSION

It is evident that the expression of class II genes is a variable, not a constant, phenomenon that depends on the immune status of the individual. The autoimmune abnormality of the BB rat that leads to the development of IDDM suggests that BB rats possess a highly activated immune system before and during the onset of IDDM. Increased numbers of class II–positive circulating T-lymphocytes have been identified in diabetes-prone and newly diabetic BB rats by cell-surface staining with monoclonal antibodies (16,17). Other studies have correlated the membrane expression of class II molecules with mRNA content of cells, indicating that the primary regulation of class II gene expression is at the level of transcription (18). It seemed reasonable that the mRNA levels of class II genes would be elevated in lymphocytes of a highly activated immune system, such as in the prediabetic BB rat. Our previous studies confirmed this hypothesis and indicated that the steady-state mRNA levels of the RT1.Dα gene were elevated in young BB rats compared with age-matched normal WF rats or older diabetic and nondiabetic BB rats (11). Thus, we extended our studies to examine the mRNA levels of class II genes of both loci, RT1.B and RT1.D, to determine if all class II gene transcripts were elevated and if there were any differences in specific class II gene transcripts in splenic lymphocytes of prediabetic and diabetic BB rats and normal rats.

We measured and compared the relative steady-state mRNA levels of the class II genes RT1.Bβ, RT1.Dα, and RT1.Dβ in splenic lymphocytes of autoimmune BB rats and age-matched histocompatible normal WF rats. Elevated mRNA levels specified by the RT1.Dα and RT1.Dβ genes, but not by the RT1.Bβ gene, were found in splenic lymphocytes of young prediabetic BB rats 45–75 days old when compared with age-matched normal WF rats or diabetic and older nondiabetic BB rats. In the diabetic and nondiabetic BB rats >75 days old, the mRNA levels of both RT1.D genes were found at a lower normal level, equivalent to the mRNA levels of the normal WF rats. In contrast, the steady-state mRNA levels of the RT1.Bβ gene were relatively constant in lymphocytes of BB and WF rats at all ages tested.

BB rats experience autoimmune pathological events to various degrees of severity, which results in the development of diabetes in a large proportion (40–60%) of the animals. The elevated RT1.Dα and RT1.Dβ mRNA levels reported here correlate with the age at which the autoimmune effector mechanisms are thought to be activated. This suggests that the elevated steady-state mRNA levels of the RT1.D genes are associated with the autoimmune disease process and provides indirect support for the RT1.D-IDDM association previously reported in the BB rat (9). These elevated steady-state mRNA levels likely reflect the proportion of splenic lymphocytes expressing the class II genes. The differences found in mRNA levels of the RT1.D genes between young prediabetic BB rats and age-matched normal WF rats or diabetic and older BB rats likely reflect differences and changes in splenic lymphocyte populations. Thus, in the prediabetic BB rats, the elevated RT1.D mRNA levels suggest the presence of highly activated splenic lymphocytes involved with the autoimmune disease process, the population number and/or activation state of which decreases after the onset of IDDM.

In addition, the elevated steady-state levels of RT1.Dα and RT1.Dβ transcripts in the prediabetic BB rats, and the relatively constant mRNA levels of the RT1.Bβ gene in all of the rats studied, suggest that the RT1.D and RT1.B class II loci are not coordinately regulated. The pattern of steady-state mRNA levels indicates that the RT1.Dα and RT1.Dβ genes are similarly regulated, but noncoordinately with the RT1.Bβ gene, in splenic lymphocytes of the BB rat during the autoimmune response.

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