Cellular and Molecular Mechanism for Kilham Rat Virus-Induced Autoimmune Diabetes in DR-BB Rats

Young-Hwa Chung, Hee Sook Jun, Mike Son, Min Bao, Hak Yeon Bae, Yup Kang and Ji-Won Yoon

*average

References This article cites 53 articles, 33 of which you can access for free at:
http://www.jimmunol.org/content/165/5/2866.full#ref-list-1

Subscription Information about subscribing to The Journal of Immunology is online at:
http://jimmunol.org/subscription

Permissions Submit copyright permission requests at:
http://www.aai.org/About/Publications/JI/copyright.html

Email Alerts Receive free email-alerts when new articles cite this article. Sign up at:
http://jimmunol.org/alerts
Cellular and Molecular Mechanism for Kilham Rat Virus-Induced Autoimmune Diabetes in DR-BB Rats

Young-Hwa Chung,* Hee Sook Jun,* Mike Son,* Min Bao,* Hak Yeon Bae,* Yup Kang, † and Ji-Won Yoon²*†

Kilham rat virus (KRV) causes autoimmune diabetes in diabetes-resistant BioBreeding (DR-BB) rats; however, the mechanism by which KRV induces autoimmune diabetes without the direct infection of β cells is not well understood. We first asked whether molecular mimicry, such as a common epitope between a KRV-specific peptide and a β cell autoantigen, is involved in the initiation of KRV-induced autoimmune diabetes in DR-BB rats. We found that KRV peptide-specific T cells generated in DR-BB rats infected with recombinant vaccinia virus expressing KRV-specific structural and nonstructural proteins could not induce diabetes, indicating that molecular mimicry is not the mechanism by which KRV induces autoimmune diabetes. Alternatively, we asked whether KRV infection of DR-BB rats could disrupt the finely tuned immune balance and activate autoreactive T cells that are cytotoxic to β cells, resulting in T cell-mediated autoimmune diabetes. We found that both Th1-like CD45RC1 CD4+ and cytotoxic CD8+b T cells were up-regulated, whereas Th2-like CD45RC− CD4+b T cells were down-regulated, and that isolated and activated CD45RC+b CD4+b and CD8+b T cells from KRV-infected DR-BB rats induced autoimmune diabetes in young diabetes-prone BioBreeding (DB-BB) rats. We conclude that KRV-induced autoimmune diabetes in DR-BB rats is not due to molecular mimicry, but is due to a breakdown of the finely tuned immune balance of Th1-like CD45RC+b CD4+b and Th2-like CD45RC− CD4+b T cells, resulting in the selective activation of β cell cytotoxic effector T cells. The Journal of Immunology, 2000, 165: 2866–2876.

Diabetes-resistant BioBreeding (DR-BB)² rats are derived from diabetes-prone forebears, which spontaneously develop a diabetic syndrome that, in many respects, resembles human type 1 diabetes (1, 2). The diabetic syndrome in diabetes-prone BioBreeding (DP-BB) rats results from the destruction of pancreatic β cells by cell-mediated immune responses (3–10). DR-BB rats do not normally develop spontaneous insulinitis and diabetes; however, when DR-BB rats were infected with Kilham rat virus (KRV), approximately one-third of the infected animals developed autoimmune diabetes similar to that found in DP-BB rats, and an additional one-third developed insulinitis without diabetes (11). If the KRV-infected rats were treated with polyinosinic:cytidylic acid (poly(I:C)), the incidence of diabetes was substantially increased to 80% (12, 13).

KRV is a member of the Parovirus family. It has a relatively simple structure comprised of linear, single-stranded DNA that encodes three structural proteins (VP1, VP2, and VP3) and two nonstructural proteins (NS1 and NS2). Part of VP2 completely overlaps the entire amino acid sequence of VP3 (14, 15). The replication of KRV takes place in the nucleus of infected cells, and the genome is not integrated with that of the cell. A striking feature of KRV is its selective replication in dividing cells (16). KRV infects lymphoid organs such as the spleen, thymus, and lymph nodes, and does not infect pancreatic β cells, indicating that KRV-induced diabetes in DR-BB rats does not result from direct cytosis of β cells after infection with the virus (17).

The mechanism by which KRV induces autoimmune type 1 diabetes without the direct infection of β cells is poorly understood. It has been suggested that molecular mimicry between KRV peptides and β cell-specific autoantigens of DR-BB rats might be a mechanism for the initiation of β cell-specific autoimmune diabetes (11, 13, 17, 18). KRV Ag-specific T cells generated by KRV infection might attack β cells if there is a common epitope between a KRV protein and an autoantigen expressed on β cells. Another possible mechanism is that KRV infection of DR-BB rats might selectively activate β cell cytotoxic effector T cells, resulting in T cell-mediated autoimmune diabetes similar to that seen in DP-BB rats. It has been suggested that KRV infection of DR-BB rats might activate silent autoantibodies in β cells, which are normally regulated by the RT6.1b subset of T cells (12). The breakdown of the immune balance by KRV infection might result in the selective activation of autoreactive T cells that are cytotoxic to β cells, leading to autoimmune diabetes in DR-BB rats.

This investigation was initiated to determine whether molecular mimicry between a KRV Ag and a β cell autoantigen or the breakdown of the finely tuned immune balance resulting in the activation of silent autoantibodies by KRV infection might be the mechanism for the KRV-induced autoimmune diabetes in DR-BB rats. We now report that recombinant vaccinia viruses (rVV) expressing KRV-specific structural and nonstructural proteins do not induce autoimmune diabetes in DR-BB rats; this suggests that...
KRV-induced diabetes in DR-BB rats is not due to molecular mimicry between KRV peptides and β cell autoantigens. In contrast, the KRV infection of DR-BB rats results in the up-regulation of β cell-specific cytotoxic CD8<sup>+</sup> and Th1-like CD45<sup>RC</sup>CD4<sup>+</sup> T cells and the down-regulation of Th2-like CD45<sup>RC</sup>CD4<sup>+</sup> T cells. In addition, the selectively activated CD45<sup>RC</sup>CD4<sup>+</sup> and CD8<sup>+</sup> T cells from KRV-infected DR-BB rats causes autoimmune diabetes in young DP-BB rats, indicating that KRV-induced diabetes in DR-BB rats is due to the breakdown of the finely tuned immune balance of T cells.

**Materials and Methods**

**Animals**

Our DR-BB and DP-BB rats were produced by breeding stocks purchased from the University of Massachusetts (DR-BB/Wor and DP-BB/Wor, Worcester, MA). The animals were maintained under specific, pathogen-free conditions at the University of Calgary (Calgary, Alberta, Canada) and used at 21–25 days of age. The use and care of the animals in this study were approved by the Animal Care Committee, Faculty of Medicine, University of Calgary.

**Cells and viruses**

The normal rat kidney (NRK), HeLa S3, BS-C-1, CV-1, and HuTK<sup>143B</sup> cell lines were obtained from the American Type Culture Collection (ATCC; Manassas, VA). NRK, HeLa S3, BS-C-1, and CV-1 cells were cultured in DMEM supplemented with 10% FCS and 5 μg/ml gentamicin, at 37°C under 5% CO<sub>2</sub>, while the HuTK<sup>143B</sup> cells were cultured in the presence of 25 μg/ml 5-bromo-2′-deoxyuridine (BrdU; Sigma, St. Louis, MO). KRV and vaccinia virus obtained from ATCC were cultivated in NRK cells and HeLa S3, respectively. The viruses were harvested from the supernatant of the infected cells at 3 days after infection at a multiplicity of infection of 1 and kept at −70°C until they were used.

**Production of rVV**

The VP1, VP2, NS1, or NS2 genes of KRV were amplified from the isolated KRV genome and cloned into the restriction enzyme site of the pGS20 vector (kindly provided by Dr. B. Moss, National Institutes of Health, Bethesda, MD) (19, 20). Briefly, a confluent monolayer of CV-1 cells (1 × 10<sup>5</sup>) were infected with wild-type vaccinia virus and then transfected with 10 μg of recombinant plasmid DNA (pGS-VP1, pGS-VP2, pGS-NS1, or pGS-NS2) using Superfectant (Qiagene, Mississauga, Ontario, Canada). As a control, the cells were transfected with the pGS20 plasmid DNA without the insert. The transfected cells were then incubated for 2 days and lysed by freezing and thawing. The rVV was isolated by plaque purification on HuTK<sup>143B</sup> cells in the presence of BrdU in an agar overlay. After several rounds of plaque purification, the virus was amplified by infecting HuTK<sup>143B</sup> cells (21). The rVV expressing either VP1, VP2, NS1, or NS2 was designated as rVV-VP1, rVV-VP2, rVV-NS1, or rVV-NS2, respectively.

**Western blotting**

To examine the production of Abs against VP1, VP2, NS1, and NS2 in rVV-VP1-, rVV-VP2-, rVV-NS1-, and rVV-NS2-infected DR-BB rats, HuTK<sup>143B</sup> cells infected with these rVV were lysed, and the cell lysate was separated on 10% SDS-PAGE and transferred to a nitrocellulose membrane. The proteins were probed with sera from rVV-VP1-, rVV-VP2-, rVV-NS1-, and rVV-NS2-infected DR-BB rats. After incubation with anti-rat IgG (1:2000 dilution), the blots were stained with horseradish peroxidase-conjugated OX-22 mAb (CD45RC; Cedarlane). The percentage of the positively stained RT6.1 subset was determined by FACS analysis of both the CD45RC<sup>−</sup> and CD45RC<sup>+</sup> cells.

**Administration of rVV to DR-BB rats**

Rats were injected i.p. with 5 μg of BrdU (5-bromo-2′-deoxyuridine) in 0.1 ml saline. The onset of diabetes was monitored by the measurement of urine glucose using a glucose stick (DiaStix, Miles, Ontario, Canada) and nonfasting blood glucose using One Touch glucometer (Lifescan, Burnaby, British Columbia, Canada) for 4 wk after virus infection. Rats that had blood glucose levels >16.7 mM (≥3 SD of the mean glucose level in uninfected DR-BB rats) were considered to be diabetic.

**In vitro T cell proliferation assay**

Splenocytes from rVV-VP1-, rVV-VP2-, rVV-NS1-, rVV-NS2-, and rVV- GS20-infected DR-BB rats were isolated at 10 days postinfection. The cells (2 × 10<sup>5</sup> cells/well) were incubated for 3 days in 200 μl of complete RPMI 1640 medium containing 10% FCS, 5 mM sodium pyruvate, 5 mM I-glutamine, 0.05 mM 2-ME, and 5 μg/ml of gentamicin in 96-well round-bottom plates (Corning Glass, Corning, NY) in the presence of inactivated, purified KRV particles (5 μg/ml) or purified recombinant NS5 proteins (5 μg/ml) and pulsed with 1 μCi [3H]thymidine (ICN, Costa Mesa, CA) 18 h before the harvest. The incorporated radioactivity was measured using a scintillation counter (Beckman, Fullerton, CA). As a control, OVA (5 μg/ml) was used.

**Immunization of DR-BB rats with rVV**

Splenocytes were isolated from DR-BB rats at 0, 3, 7, and 10 days after KRV infection and incubated for 30 min at 4°C with FITC-conjugated OX-19 mAb (CD5; Cedarlane, Hornby, Ontario, Canada), PE-conjugated OX-35 mAb (CD4; Pharmingen, San Diego, CA), or PE-conjugated OX-8 mAb (CD8; Cedarlane). The cells were then washed and analyzed by FACScan (Becton Dickinson, Sunnyvale, CA), as described elsewhere (22). To evaluate the CD45RC<sup>−</sup> subset population, splenocytes isolated from DR-BB rats at 7 days after KRV infection were stained with biotin-conjugated OX-22 mAb (CD45RC; Cedarlane) and PE-conjugated OX-35 mAb for 30 min at 4°C. After washing, streptavidin-Red-613 (Life Technologies) was added to label biotin. Splenocytes isolated from PBS-treated animals were used as a control. To analyze the RT6.1 subset population, splenocytes from DR-BB rats were isolated at 7 days after KRV infection and stained with PE-conjugated OX-22 mAb and FITC-conjugated RG7/11.1 mAb (anti-rat IgG2b; Pharmingen) was added to label the P4/16 mAb. The percentage of the positively stained RT6.1 subset was determined by FACS analysis of both the CD45RC<sup>−</sup> and CD45RC<sup>+</sup> T cell populations.

**Isolation of CD4<sup>+</sup>, CD8<sup>+</sup>, CD45RC<sup>−</sup> CD4<sup>+</sup>, and CD45RC<sup>−</sup> CD4<sup>+</sup> T lymphocyte subpopulations**

Splenocytes were prepared from DR-BB rats at 7 days after KRV infection and T cells were enriched using an immunomunotum of T cells (Cedarlane) according to the manufacturer’s protocol. To isolate CD<sup>+</sup> and CD<sup>+</sup> T cells, the enriched T cells were incubated with FITC-conjugated OX-8 mAb and PE-conjugated OX-35 mAb. To isolate CD45RC<sup>−</sup> CD<sup>+</sup> and CD45RC<sup>−</sup> CD4<sup>+</sup> T cells, the enriched T cells were incubated with negative selection (23) and incubated with FITC-conjugated OX-22 mAb and PE-conjugated OX-35 mAb at 4°C for 30 min. The labeled cells were sorted using FACStarPlus (Becton Dickinson), and the purity of the sorted cells was analyzed by FACScan using FITC-conjugated OX-22 mAb and PE-conjugated OX-35 mAb.

**Measurement of the proliferative T cells by BrdU incorporation**

The measurement of incorporated BrdU in the DNA from the proliferating T cell subsets was performed as described elsewhere (24, 25). Briefly, DR-BB rats were injected i.p. with BrdU (5 μg/g body weight) administered for 3 consecutive days. As an additional group, all four rVV-VP1-, rVV-VP2-, rVV-NS1-, and rVV-NS2- (5 × 10<sup>5</sup> PFU/rat each rVV/rat) were combined and administered. The onset of diabetes was monitored by the measurement of urine glucose using a glucose stick (DiaStix, Miles, Ontario, Canada) and nonfasting blood glucose using One Touch glucometer (Lifescan, Burnaby, British Columbia, Canada) for 4 wk after virus infection. Rats that had blood glucose levels >16.7 mM (≥3 SD of the mean glucose level in uninfected DR-BB rats) were considered to be diabetic.
with 2% paraformaldehyde supplemented with 0.1% Tween 80 (Sigma) at 4°C overnight. The cells were washed, pelleted, and incubated with 50 Kunitz units of DNase I (pH 5.0; Sigma). After washing, the cells were incubated with FITC-conjugated anti-BrdU mAb (Becton Dickinson) and the BrdU3+ T cells were measured on a FACScan. For measurement of proliferating CD45RC+CD4+ and CD45RC+CD4+ T populations, the splenocytes were stained with PE-conjugated OVA-35 mAb and biorin-conjugated OX-22 mAb, and the biotin was stained with streptavidin-conjugated Red-613. After permeabilization, the cells were stained with FITC-conjugated anti-BrdU mAb and analyzed by three-color analysis.

**RT-PCR analysis of cytokine and KRV gene expression**

The total RNA was extracted from the cells by the acid guanidin thiocyanate phenol-chloroform method (26). Three micrograms of the total RNA was converted to cDNA using Superscript II (Life Technologies), and PCR was performed using specific primers for several cytokines (27–30) and the HPRT signal. After amplification, the products were subjected to ethidium bromide staining. The amplified signal was analyzed by densitometric analysis using NIH Image 1.6 software and normalized by the HPRT signal.

**Administration of OX-8 mAb to KRV and poly(I:C)-treated DR-BB rats**

To deplete the CD8+ T cells, OX-8 mAb was administered i.p (500 μg/400 μl PBS) 2 days before the injection of rats with KRV and, subsequently, poly(I:C) was administered i.p for 3 days. After the initial injection, OX-8 mAb was administered twice per week for up to 28 days postinfection. The depletion of CD8+ T cells from the spleen was confirmed by FACS analysis (1.2 ± 0.4% in OX-8 mAb-treated rats vs 15.5 ± 3.2% in PBS-treated control rats). The onset of diabetes was monitored using urine glucose sticks and confirmed by the measurement of blood glucose for 4 wk following infection (22). As a control, DR-BB rats were treated with 400 mg of PBS.

**Histologic examination**

Pancreata were fixed with formalin, paraffin-embedded, serially sectioned at 5 μm, and stained with hematoxylin and eosin. The infiltrated islets (20–25 islets/animal) were classified as early, intermediate, late, or end-stage insulitis based on immunohistochemical examination and confirmed by the measurement of blood glucose for 4 wk following infection (22). As a control, DR-BB rats were treated with 400 mg of PBS.

**Adaptive transfer of diabetes**

Splenocytes were isolated from uninfected DR-BB rats or nondiabetic animals treated with rVV or KRV at 28 days after infection and were incubated at a concentration of 2 × 10^5 cells/ml in complete RPMI 1640 in the presence of 5 μg/ml Con A (Pharmacia Biotech, Uppsala, Sweden) for 3 days at 37°C under 5% CO2. The cells were washed three times with RPMI 1640, and viable spleen cells (1–5 × 10^5 cells/rat) were injected into 21- to 25-day-old DP-BB rats. After the transfer of spleen cells, the animals were monitored every other day for 4 wk for glycosuria (>2+) and hyperglycemia (>16.7 mM) as described elsewhere (8, 22).

For the transfer of CD4+, CD8+, or CD45RC-CD4+ T cells, these T cell subpopulations were isolated by negative selection as described previously (23). CD8+ T cells were isolated by incubation of the splenocytes with the mAbs OX-12 (Ig κ; Cedarlane), OX-42 (CD11b and c; Cedarlane), OX-6, and W3/25 (CD4; Cedarlane). CD4+ T cells were isolated by incubation of splenocytes with the mAbs OX-12, OX-43, OX-6, and OX-8, and CD45RC+CD4+ T cells were selected with the same Ab mixture and OX-22. CD45RC+CD4+ T cells were positively selected using OX-22 mAb after isolation of CD4+ T cells. The T cell subpopulations were stimulated with Con A (5 μg/ml) for 5 days, and viable CD4+ T cells (5 × 10^6 cells/rat), CD45RC+CD4+ T cells (5 × 10^6 cells/rat), CD45RC-CD4+ T cells (5 × 10^5 cells/rat) with CD8+ T cells (5 × 10^6 cells/rat), or each of these cell populations alone were transfused to young DP-BB rats. The recipient DP-BB rats were monitored for the onset of diabetes as described above.

**ELISA for anti-OVA Abs**

DR-BB rats were treated with PBS or KRV (1 × 10^5 TCID50/rat) and then immunized s.c. with OVA (400 μg; Sigma) in IFA at 1 day after treatment and boosted at 8 days after treatment. Sera were collected at 7 days after primary and secondary immunization. ELISA was performed with serial dilutions of sera to detect anti-OVA Abs. The plates (Corning) were coated with OVA Ag (20 μg/ml/100 μl in 0.1 M NaHCO3, pH 9.3) and incubated at 4°C overnight. The plates were blocked with 2% BSA in PBS, washed with PBS containing 0.5% Tween 80, and incubated with the diluted sera at room temperature for 1 h. The plates were incubated with alkaline phosphatase-conjugated anti-rat IgG1 or anti-rat IgG2a (PharMingen). After the addition of 200 μl of p-nitrophenyl-phosphate (Sigma), the plates were incubated in the dark at room temperature for 30 min, followed by reading at 405 nm on a microtiter plate reader.

**Statistical analysis**

Statistical analysis was performed using Fisher’s exact test or the Student’s t test. Values of p < 0.05 were considered to be significant.

**Results**

**KRV proteins failed to induce diabetes in DR-BB rats**

To determine whether T cells generated against KRV proteins can cross-react with β cell Ags by a shared epitope and destroy β cells, we produced an rVV expressing the capsid proteins VP1 or VP2 of KRV (rVV-VP1 or rVV-VP2). DR-BB rats (21–25 days old) were injected with poly(I:C) to increase the incidence of diabetes (12, 13) and infected with rVV-VP1 or rVV-VP2 to generate T cells against KRV-VP1 or -VP2 protein. We found that KRV-VP1 and -VP2 mRNA was clearly expressed in the splenocytes of DR-BB rats infected with rVV-VP1 or rVV-VP2, respectively (Fig. 1A). We then examined the production of Abs against VP1 and VP2 by Western blot to find whether the expression of VP1 or VP2 was sufficient to induce humoral immune responses against the KRV-VP1 and -VP2 proteins. We found strong humoral responses.

---

**Table I. Oligonucleotide primers of the KRV genome and PCR conditions**

<table>
<thead>
<tr>
<th>Primer Sequences</th>
<th>Annealing Temperature</th>
<th>Fragment Size (bp)</th>
</tr>
</thead>
</table>
| VP1 5’ GATTTATGCGCATTCTGAAAAAG
| 3’ CTCAAGTGGTCAAGAACTTT | 58°C | 403 |
| VP2 5’ GTTCTGTACAGGCTTATGAT
| 3’ GTGGATACGCTGTTTAAAT | 55°C | 488 |
| NS1 5’ GCCAGAATCCACTGGAAGAG
| 3’ AGAGAAGCTTGGTGCAAAAGCTTG | 64°C | 1,556 |
| NS2 5’ ATGCCGTGGAAAGCTTACCC
| 3’ TTCTCGGGAGGTGCCATCGTA | 60°C | 658 |
evidenced by the induction of high titers of Abs against VP1 and VP2 (Fig. 1B). Furthermore, we found that VP1- and VP2-specific T cells were clearly generated (Fig. 1C), but none of the DR-BB rats infected with rVV-VP1 or poly(I:C) or rVV-VP2 and poly(I:C) developed diabetes, whereas 80% of DR-BB rats infected with KRV and poly(I:C) became diabetic (Table II). When we examined the development of insulitis in rVV-VP1 and poly(I:C)-infected DR-BB rats and rVV-VP2 and poly(I:C)-infected DR-BB rats, we found that none of the DR-BB rats developed insulitis (data not shown). These results indicate that rVV-VP1 and -VP2 can induce humoral and cellular immune responses against the viral proteins, but T cells generated against KRV-VP1 and -VP2 do not attack pancreatic β cells. Similarly, rVVs expressing the nonstructural proteins, NS1 or NS2, failed to induce insulitis and diabetes in DR-BB rats (Table II). To determine whether the T cell responses against multiple KRV Ags are needed to induce the development of diabetes, we administered rVV-VP1, -VP2, -NS1, or rVV-NS2 to DR-BB rats followed by poly(I:C) treatment. We found that none of the DR-BB rats that were infected with all four rVVs expressing KRV proteins developed diabetes (Table II).

To determine whether Con A-stimulated splenocytes from rVV-infected DR-BB rats have the ability to transfer diabetes, we isolated splenocytes from rVV-infected DR-BB rats, stimulated them with Con A for 3 days, and transferred them to young DP-BB rats. We found that none of the DP-BB rat recipients developed diabetes, whereas splenocytes from nondiabetic, KRV-infected DR-BB rats induced diabetes in young DP-BB rat recipients, indicating that T cell responses against multiple KRV Ags are needed to induce the development of diabetes.

Table II. rVV-expressing KRV proteins failed to induce autoimmune diabetes in DR-BB rats

<table>
<thead>
<tr>
<th>Treatment of Donor</th>
<th>Incidence of Diabetes in Donor (%)</th>
<th>Incidence of Diabetes in Recipient (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>KRV</td>
<td>33 (6/18)</td>
<td>75 (6/8)</td>
</tr>
<tr>
<td>KRV + poly (I:C)</td>
<td>80 (8/10)</td>
<td>ND</td>
</tr>
<tr>
<td>rVV-VP1</td>
<td>0 (0/6)</td>
<td>0 (0/6)</td>
</tr>
<tr>
<td>rVV-VP1 + poly (I:C)</td>
<td>0 (0/6)</td>
<td>0 (0/6)</td>
</tr>
<tr>
<td>rVV-VP2</td>
<td>0 (0/6)</td>
<td>0 (0/6)</td>
</tr>
<tr>
<td>rVV-VP2 + poly (I:C)</td>
<td>0 (0/6)</td>
<td>0 (0/6)</td>
</tr>
<tr>
<td>rVV-NS1</td>
<td>0 (0/6)</td>
<td>0 (0/6)</td>
</tr>
<tr>
<td>rVV-NS1 + poly (I:C)</td>
<td>0 (0/6)</td>
<td>0 (0/6)</td>
</tr>
<tr>
<td>rVV-NS2</td>
<td>0 (0/6)</td>
<td>0 (0/6)</td>
</tr>
<tr>
<td>rVV-NS2 + poly (I:C)</td>
<td>0 (0/6)</td>
<td>0 (0/6)</td>
</tr>
<tr>
<td>rVV-VP1/VP2/NS1/NS2 + poly (I:C)</td>
<td>0 (0/8)</td>
<td>0 (0/8)</td>
</tr>
</tbody>
</table>

* DR-BB rats (21–25 days old) were infected with rVV-VP1, rVV-VP2, rVV-NS1, or rVV-NS2 (1 × 10^5 PFU) singly, or a combination of all rVVs (5 × 10^4 PFU/rVV). KRV (1 × 10^5 TCID_{50}) was injected into age-matched DR-BB rats as a positive control. Poly (I:C) (5 μg of body weight) was administered three consecutive days after injection of the viruses. The onset of diabetes was monitored with a urine glucose test for 4 wk postinfection and confirmed by measurement of blood glucose (22).

* Donor splenocytes from nondiabetic rats of each group were isolated at 28–30 days after virus infection. Splenocytes were activated with Con A (5 μg/ml) for 3 days and 3–5 × 10^7 cells were transferred to 21- to 25-day-old DP-BB rats. The onset of diabetes was monitored with a urine glucose test for 4 wk posttransfer and confirmed by measurement of blood glucose (22).

* p < 0.001 when compared with the group that received splenocytes from DR-BB rats infected with KRV.

![FIGURE 1](https://www.jimmunol.org/)  Cellular and humoral immune responses developed against structural proteins (VP1 and VP2 of KRV) in rVV-VP1- or rVV-VP2-infected DR-BB rats. A. Splenocytes were isolated from DR-BB rats (n = 4/group; representative data from two animals shown) on different days after infection with rVV-VP1 or rVV-VP2 (1 × 10^7 PFU). VP1 or VP2 expression was analyzed by RT-PCR. Lanes 1 and 4, Splenocytes from DR-BB rats infected with control rVV at 14 days postinfection. Lanes 2 and 3, Splenocytes from Dr-BB rats infected with rVV-VP1 at 3 and 14 days postinfection, respectively. Lanes 5 and 6, Splenocytes from DR-BB rats infected with rVV-VP2 at 3 and 14 days postinfection, respectively. As an internal standard, HPRT mRNA was amplified. B. Induction of Abs against the VP1 or VP2 protein in rVV-VP1- or rVV-VP2-infected DR-BB rats. HuTK^− 143B cells infected with control rVV (lane 1, upper and lower panels), rVV-VP1 (lane 2, upper panel), or rVV-VP2 (lane 2, lower panel) were lysed and reacted with pooled sera (n = 4/group) from DR-BB rats infected with rVV-VP1 (upper panel) or rVV-VP2 (lower panel). C. Splenocytes from rVV-VP1-, rVV-VP2-, or control rVV (rVV-CON)-infected DR-BB rats (n = 4/group) were isolated at 10 days postinfection. The splenocytes (2 × 10^5 cells/200 μl/well) were cultured with or without inactivated KRV particles (5 μg/ml) for 3 days. Proliferation was determined by [3H]thymidine uptake. OVA was used as a control Ag. Error bars are SEM. * p < 0.001 when compared with control rVV-infected group.
that T cells generated against KRV proteins fail to induce autoimmune diabetes (Table II).

**KRV-specific structural proteins, but not nonstructural proteins, can prevent KRV-induced diabetes, and infectious KRV is absolutely required for the induction of autoimmune diabetes in DR-BB rats**

To determine whether KRV-specific proteins can prevent the development of KRV-induced autoimmune diabetes in DR-BB rats by neutralizing infectious KRV with Abs against the KRV-specific peptides, we immunized DR-BB rats with rVV-VP1, rVV-VP2, rVV-NS1, or rVV-NS2 and then treated them with KRV and poly(I:C) 1 wk later. We found that none of the rVV-VP1 (0/10-) or rVV-VP2 (0/10-) immunized DR-BB rats developed diabetes, whereas 90% (9/10) of the rVV-NS1-immunized and 70% (7/10) of the rVV-NS2-immunized DR-BB rats developed diabetes when they were subsequently treated with KRV and poly(I:C). The incidence of diabetes in the rVV-NS1- and rVV-NS2-immunized rats was similar to the incidence found in nonimmunized KRV and poly(I:C)-treated rats (80%, Fig. 2A). When we examined the pancreatic islets from rVV-VP1- and rVV-VP2-immunized rats, most of the islets were intact. In contrast, the majority of the islets from rVV-NS1- or rVV-NS2-immunized rats showed severe insulitis (Fig. 2, B and C). When we examined the presence of the KRV genome in the spleen, thymus, and lymph nodes of rVV-VP1- and rVV-VP2-immunized rats, we found no evidence of the KRV genome in the tissues (data not shown), indicating that a sufficient amount of neutralizing Ab against KRV was produced in these immunized rats. When we immunized DR-BB rats with the inactivated KRV particles, results similar to that found in rVV-VP1- and -VP2-infected rats were observed (data not shown), indicating that infectious KRV, rather than viral specific proteins, is absolutely required for the induction of autoimmune diabetes in DR-BB rats.

**KRV infection of DR-BB rats results in preferential proliferation of CD8+ T cells as compared with CD4+ T cells**

We first determined whether the infection of DR-BB rats with KRV results in a change in the total number of spleen cells by counting the number of single cells from spleen using the trypan blue exclusion method. The number of splenocytes from KRV-infected DR-BB rats increased gradually after KRV infection and reached 1.4-fold (p < 0.01) and 1.5-fold (p < 0.01) by 7 and 10 days postinfection, respectively, as compared with cell numbers from PBS-treated DR-BB rats (data not shown). We next determined which subpopulation of splenic T cells (CD4+ or CD8+) is altered after infection of DR-BB rats with KRV by two-color flow cytometric analysis. We found that the percentage of CD8+ T cells gradually increased and reached 18.5 ± 2.1% at 10 days postinfection (PBS-treated control: 12.0 ± 1.5%) (Fig. 3A). When we examined the relationship of CD8+ T cells with CD4+ T cells, we found that the ratio of CD8+ to CD4+ T cells increased from 0.13 ± 0.02 (PBS-treated control) to 0.008 ± 0.001 at 10 days postinfection (PBS-treated control: 0.13 ± 0.02) (Fig. 3B).
To determine whether this preferential proliferation of CD8\(^+\) T cells is correlated with more efficient replication of KRV in CD8\(^+\) T cells as compared with CD4\(^+\) T cells, we isolated T cells from DR-BB rats at 7 days after KRV infection, sorted the CD4\(^+\) and CD8\(^+\) T cell subsets, and extracted the RNA. We then examined KRV-NS1 gene expression in these T cell subsets by RT-PCR, as several lines of evidence have shown that the NS1 protein of parvoviruses is essential for parvoviral DNA replication (31, 32). We found that the KRV gene was preferentially expressed in CD8\(^+\) T cells as compared with CD4\(^+\) T cells during the early phase of KRV infection (Fig. 3C). We also measured the incorporation of BrdU into the proliferating T cell subsets from KRV-infected DR-BB rats to determine whether CD8\(^+\) T cells preferentially divide as compared with CD4\(^+\) T cells. We found that more CD8\(^+\) T cells than CD4\(^+\) T cells were BrdU\(^+\), indicating that CD8\(^+\) T cells are more proliferative than CD4\(^+\) T cells during the early phase of KRV infection (Fig. 3D).

Depletion of CD8\(^+\) T cells results in a significant decrease in the incidence of KRV-induced diabetes in DR-BB rats

To determine whether CD8\(^+\) T cells are involved in the development of KRV-induced autoimmune diabetes in DR-BB rats, we administered mAb against CD8\(^+\) T cells to KRV and poly(I:C)-treated DR-BB rats. Three of 11 (27%) mAb-treated DR-BB rats became diabetic. All of the six isotype-matched anti-mouse IgG-treated DR-BB rats (6/6; 100%) and 7 of 9 (78%) of the PBS-treated DR-BB rats developed diabetes (Table III). When we examined the pancreatic islets from the anti-CD8\(^+\) T cell Ab (OX-8)-treated DR-BB rats, we found that 34% of the examined islets were intact, 22% exhibited early-stage insulitis, 18% showed intermediate-stage insulitis, and 16% exhibited late-stage insulitis (Table IV). None of the islets examined from the PBS-treated DR-BB rats injected with KRV and poly(I:C) were intact, 2% of the examined islets exhibited early-stage insulitis, 6% showed...
Th1-like CD4 + T cells are up-regulated whereas Th2-like CD4 + T cells are down-regulated in DR-BB rats during acute KRV infection

The percentage of CD4 + T cells in DR-BB rats gradually decreased after KRV infection as compared with that of CD8 + T cells. To determine which CD4 + T cell subset, Th1 and/or Th2, was decreased after KRV infection, we measured the percentage of Th1-like, CD45RC + CD4 + T cells and Th2-like, CD45RC − CD4 + T cells in the splenocytes of DR-BB rats at 7 days after KRV infection. In rats, Th1- and Th2-like cells are defined by the expression of CD45RC. Th1-like cells express high CD45RC, and Th2-like cells express low CD45RC. We found that the percentage of CD45RC⁺CD4 + T cells decreased to 11.4 ± 0.7% (p < 0.05) compared with PBS-treated controls (24.7 ± 0.7%), whereas the percentage of CD45RC⁻CD4 + T cells increased to 15.0 ± 0.6% (p < 0.05) compared with that of PBS-treated controls (9.3 ± 0.3%) (Fig. 4A). To confirm that CD45RC⁺CD4 + and CD45RC − CD4 + T cells are Th1-like and Th2-like cells, respectively, in this animal model, we examined the expression of IFN-γ and IL-4 in the sorted CD45RC⁺CD4 + and CD45RC − CD4 + T cells from KRV-infected DR-BB rats using RT-PCR. We found that CD45RC⁺CD4 + T cells showed a higher expression of IFN-γ (a Th1 cytokine) and a lower expression of IL-4 (a Th2 cytokine), whereas CD45RC − CD4 + T cells showed a higher expression of IL-4 and a lower expression of IFN-γ (Fig. 4B). This result indicates that CD45RC⁺CD4 + T cells are Th1-like cells and CD45RC − CD4 + T cells are Th2-like cells in the DR-BB rat.

To determine which subset of CD4 + T cells is preferred for the amplification of the KRV gene, we examined the expression of the KRV-NS1 gene by RT-PCR in sorted CD45RC⁺CD4 + and CD45RC − CD4 + T cells after KRV infection. We found that the expression of the KRV gene was significantly higher in CD45RC⁺CD4 + than in CD45RC − CD4 + T cells (Fig. 4B). To determine whether CD45RC⁺CD4 + T cells are more proliferative than CD45RC − CD4 + T cells, we measured the percentage of BrdU + cells in CD45RC⁺CD4 + and CD45RC − CD4 + T cells from the splenocytes of KRV-infected DR-BB rats and vaccinia virus-infected or PBS-treated DR-BB rats as controls. As shown in Fig. 5A, the ratio of proliferative CD45RC⁺CD4 + to CD45RC − CD4 + T cells increased in the splenocytes of KRV-infected DR-BB rats (2.2 ± 0.2, p < 0.05 compared with the PBS-treated group). However, this ratio decreased in the splenocytes of vaccinia virus-infected DR-BB rats (1.3 ± 0.1) compared with that in PBS-treated group (1.7 ± 0.1). To correlate the greater proliferation of Th1-like cells with a biased activation of Th1-like cells during KRV infection, we enriched CD4 + T cells and measured the expression of IFN-γ and IL-4 at 7 days after KRV or vaccinia virus infection (Fig. 5B). The ratio of IFN-γ mRNA to IL-4 mRNA expression in CD4 + T cells from KRV-infected DR-BB rats increased >10- and 3-fold compared with that in CD4 + T cells from PBS-treated and vaccinia virus-infected DR-BB rats, respectively (Fig. 5C). Taken together, these results suggest that KRV infection of DR-BB rats selectively activate Th1-like CD4 + cells and down-regulate Th2-like CD4 + T cells.

To confirm that KRV selectively activates Th1-like CD4 + T cells, we measured the titer of the IgG isotypes, IgG2a and IgG1, as representatives of the Th1- and Th2-type immune response, respectively, in KRV-infected DR-BB rats after primary immunization with OVA. We found that KRV-infected rats showed a higher titer of the IgG2a isotype (OD = 1.5 ± 0.5) than the IgG1 isotype (OD = 0.3 ± 0.2). In contrast, PBS-treated control rats showed no significant difference between the titer of IgG1 (OD = 0.8 ± 0.3) and IgG2a (OD = 0.4 ± 0.2). After secondary immunization of DR-BB rats with OVA, KRV-infected rats showed an increase in the titer of the IgG1 isotype, but no increase in the IgG2a isotype. In contrast, PBS-treated rats showed a dramatic increase in both IgG1 and IgG2a production (Fig. 6). These results suggest that KRV infection induces a strong bystander Th1-biased
activation, and these activated Th1-like T cells may be more susceptible to anergy, resulting in the failure of the secondary immunization to boost the Th1 immune response.

**KRV-activated CD45RC⁺ CD4⁺ Th1-like T cells and CD8⁺ T cells can induce autoimmune diabetes in young DP-BB rats**

To determine whether the activated Th1-like CD45RC⁺ CD4⁺ and CD8⁺ T cells are directly involved in the development of autoimmune diabetes, we isolated CD45RC⁺ CD4⁺ and CD8⁺ T cells from KRV-infected DR-BB rats at 4 wk after infection, stimulated the cells with Con A, and transferred them to young DP-BB recipients. We found that 88% of DP-BB rats that received both Th1-like CD45RC⁺ CD4⁺ and CD8⁺ T cells developed diabetes (Table V). There was no significant difference in the incidence of diabetes between DP-BB rats that received the CD45RC⁺ CD4⁺ and CD8⁺ T cells and those that received total splenocytes. This result indicates that CD45RC⁺ CD4⁺ Th1-like T cells and CD8⁺ T cells activated by KRV infection are major effector T cells that can induce autoimmune diabetes. However, the incidence of diabetes in DP-BB rats that received either CD4⁺, CD8⁺, or CD45RC⁺ CD4⁺ T cells alone was significantly decreased as compared with that found in rats that received combined CD45RC⁺ CD4⁺ and CD8⁺ T cells (Table V). In addition, the incidence of diabetes (3/6) in recipients of a combination of CD8⁺ T cells from infected rats and CD45RC⁺ CD4⁺ T cells from uninfected rats was similar to that in recipients of CD8⁺ T cells alone from infected rats (3/6). The incidence of diabetes (2/6) in recipients of a combination of CD45RC⁺ CD4⁺ T cells from infected rats and CD8⁺ T cells from uninfected rats was the same as that in recipients of CD45RC⁺ CD4⁺ T cells alone from infected rats (2/6). These results indicate that CD45RC⁺ CD4⁺ and CD8⁺ T cells activated by KRV infection work synergistically to destroy pancreatic β cells. In contrast, none of the DP-BB rats that received a combination of CD45RC⁺ CD4⁺ Th2-like T cells and CD8⁺ T cells or CD45RC⁺ CD4⁺ T cells alone developed diabetes (Table V). In addition, 63% of the DP-BB rats that received both CD4⁺ and CD8⁺ T cells developed diabetes (Table V). The incidence of diabetes in these recipients was slightly lower than that found in the DP-BB rats that received both CD45RC⁺ CD4⁺ and CD8⁺ T cells. These results indicate that CD45RC⁺ CD4⁺ T cells may hinder CD8⁺ T cell-mediated β cell destruction.

**Discussion**

KRV-induced autoimmune diabetes in DR-BB rats is known to be one of the best animal models for studies on virus-induced autoimmune diabetes in humans. However, the precise mechanism by which KRV induces autoimmune diabetes without the direct infection of pancreatic β cells was not known. Molecular mimicry, such as a common epitope between a KRV-specific peptide and a β cell autoantigen, has been suggested as a mechanism for the initiation of β cell-specific autoimmune diabetes (11, 13, 17, 18). If molecular mimicry is involved in the initiation of β cell-specific autoimmune, then KRV Ag-specific T cells generated by KRV peptides might cross-react with pancreatic β cells and attack them, resulting in the development of insulitis and, subsequently, diabetes. To test this hypothesis, we used rVVs expressing KRV proteins, because the wild-type strain of vaccinia virus does not induce insulitis or diabetes in DR-BB rats (11), and rVVs have been used as a vehicle for the expression of foreign proteins that successfully induced humoral and cell-mediated immune responses (33–35). We constructed rVVs expressing the KRV peptides VP1, VP2, NS1, or NS2. However, we did not construct an rVV expressing VP3, because the amino acid sequence of the KRV peptide VP3 completely overlaps that of the VP2 peptide. When we infected DR-BB rats with the rVVs expressing the KRV peptides, we found that each viral peptide was clearly expressed in the infected DR-BB rats, viral peptide-specific T cells were generated, and Abs against the KRV-peptides were also induced. However, none of the DR-BB rats developed insulitis or diabetes. This result indicates that molecular mimicry between KRV peptides and β peptides might cross-react with pancreatic β cell autoantigens.

**FIGURE 4.** Th1-like CD4⁺ T cells are up-regulated whereas Th2-like CD4⁺ cells are down-regulated in DR-BB rats during acute KRV infection.

A. Splenocytes were isolated from DR-BB rats at 7 days after KRV infection or PBS-treatment (n = 4/group), labeled with biotin-conjugated OX-22 and streptavidin Red-613, and PE-conjugated OX-35 mAb, and analyzed on a flow cytometer. Representative data from two different experiments are shown. B. After enrichment of the CD4⁺ T cells, the cells were sorted into CD45RC⁺ CD4⁺ and CD45RC⁺ CD4⁺ T cells. The purity of the sorted cells was examined using FACScan after staining of the cells with FITC-conjugated OX-22 mAb and PE-conjugated OX-35 mAb. Cytokine and KRV-NS1 gene expression were measured by RT-PCR. Lane M, 100-bp DNA ladder for cytokine gene expression and 1-kb DNA ladder for KRV-NS1 gene expression. Lane 1, CD45RC⁺ CD4⁺ T cells. Lane 2, CD45RC⁺ CD4⁺ T cells.
cell-specific autoantigens in DR-BB rats is unlikely to be a mechanism by which KRV induces cytokine diabetes.

Because the KRV proteins failed to induce autoimmune diabetes in DR-BB rats, we asked alternatively whether KRV infection of DR-BB rats could disturb the finely tuned immune balance and activate autoreactive T cells that are cytotoxic to β cells, resulting in T cell-mediated autoimmune diabetes similar to that seen in DP-BB rats. We first examined the CD4⁺ and CD8⁺ T cell populations in the splenocytes of DR-BB rats after KRV infection. We found that the percentage of CD8⁺ T cells increased significantly, whereas the percentage of CD4⁺ T cells decreased, although the absolute number of both CD4⁺ and CD8⁺ T cells was increased during KRV infection. In addition, CD8⁺ T cells preferentially proliferated as compared with CD4⁺ T cells in KRV-infected DR-BB rats. When we treated KRV-infected DR-BB rats with OX-8 mAb, the incidence of diabetes in these rats was significantly decreased, indicating that CD8⁺ T cells are clearly involved in the destruction of β cells. It has been reported that the treatment of DP-BB rats with anti-NK cell Ab failed to prevent diabetes, while OX-8 mAb treatment successfully prevented diabetes (36). Therefore, it is more likely that CD8⁺ T cells may play a major role in KRV-induced diabetes, although we cannot absolutely exclude the possibility of the involvement of NK cells, because OX-8 mAb also depletes NK cells.

In the rat, CD4⁺ T cells can be divided into Th1-like CD45RC⁺ CD4⁺ T cells, which express IL-2 and IFN-γ and play an important role in cell-mediated immune responses, and Th2-like CD45RC⁻ CD4⁺ T cells, which express IL-4 and IL-10 and play an important role in humoral immune responses (37). The immune balance between Th1- and Th2-type cells has been suggested to play an important role in the maintenance of peripheral tolerance. The dominance of Th1 cells over Th2 cells is associated with the development of autoimmune insulin-dependent diabetes mellitus (IDDM), whereas the dominance of Th2 cells over Th1 cells is associated with the prevention of IDDM (38–40). The transfer
of Th1-like CD45RC⁺CD4⁺ T cell subsets into congenic athymic nude rats induces a wasting disease with inflammatory infiltrates into a variety of organs (41). In contrast, Th2-like CD45RC⁺CD4⁺ T cells have been shown to suppress the progression of various autoimmune diseases including IDDM (42), inflammatory bowel disease (43), a wasting disease (44), and autoimmune graft-vs-host disease (45). Previously, we found that KRV infection in DR-BB rats increased the expression of Th1-type cytokines in the splenocytes and pancreatic infiltrates (13). Therefore, we asked whether the proportions of Th1 and Th2 cells were altered during KRV infection in DR-BB rats. We found that the numbers of Th2-like CD45RC⁺CD4⁺ T cells were significantly decreased and Th1-like CD45RC⁺CD4⁺ T cells were significantly increased in the splenocytes of KRV-infected DR-BB rats compared with PBS-treated controls. To confirm that the CD45RC⁺CD4⁺ T cells were Th1-like cells and CD45RC⁺CD4⁺ T cells were Th2-like cells, we examined the expression of IFN-γ and IL-4 and found that CD45RC⁺CD4⁺ T cells expressed higher amounts of IFN-γ than CD45RC⁺CD4⁺ T cells, and CD45RC⁻CD4⁺ T cells expressed higher amounts of IL-4 than CD45RC⁺CD4⁺ T cells. In addition, we examined the expression of TGF-β, as TGF-β is known to be a suppressor cytokine, and TGF-β produced by a T cell clone is responsible for the prevention of autoimmune diseases such as autoimmune encephalomyelitis (46), inflammatory bowel disease (47), and autoimmune diabetes (48). We found that TGF-β was also highly expressed in CD45RC⁺CD4⁺ T cells from KRV-infected DR-BB rats, as compared with the expression in CD45RC⁺CD4⁺ T cells.

KRV infection induced a strong Th1-biased immune response; therefore, we postulated that the production of an IgG2a isotype, which represents a Th1 response, would be higher in KRV-infected DR-BB rats after primary immunization with OVA. As expected, the titer of the IgG2a isotype of anti-OVA Abs was highly increased in KRV-infected DR-BB rats. This result indicates that the activated Th1-like cells may be energized in response to the secondary immunization, because Th1-type T cells are more susceptible to anergy and activation-induced cell death, whereas Th2-type T cells are more resistant (49–53).

It appears to be clear that infection of DR-BB rats with KRV results in the selective activation of Th1-like CD45RC⁺CD4⁺ T cells and CD8⁺ T cells. Thus, we asked whether the selectively activated Th1-like CD45RC⁺CD4⁺ and CD8⁺ T cells could induce autoimmune diabetes in young DP-BB rats. We isolated CD45RC⁺CD4⁺ and CD8⁺ T cells from DR-BB rats after infection with KRV, stimulated the isolated cells with Con A, and transferred them to young DP-BB rats. Eighty-eight percent of the recipients of both CD45RC⁺CD4⁺ and CD8⁺ T cells developed autoimmune diabetes, indicating that CD45RC⁺CD4⁺ and CD8⁺ T cells are major effector T cells that can induce autoimmune diabetes. However, the incidence of diabetes in DP-BB rats that received either CD45RC⁺CD4⁺ or CD8⁺ T cells alone was significantly decreased as compared with that found in rats that received a combination of CD45RC⁺CD4⁺ and CD8⁺ T cells, and a combination of CD8⁺ or CD45RC⁺CD4⁺ T cells from infected rats did not change the incidence of diabetes, indicating that Th1-like CD44⁺ and CD8⁺ T cells from KRV-infected rats work synergistically to destroy pancreatic β cells, as proposed previously (13). In contrast, none of the recipients of both CD45RC⁺CD4⁺ and CD8⁺ T cells developed diabetes, indicating that CD45RC⁺CD4⁺ T cells play a role as regulatory T cells. However, the incidence of diabetes in recipients of a combination of CD4⁺ and CD8⁺ T cells was not significantly different from that in the recipients of CD8⁺ and CD45RC⁺CD4⁺ T cells, probably due to the shifted balance toward diabetogenic effectors in the CD4⁺ T cell population. Then, we examined whether CD45RC⁺CD4⁺ T cells express RT6.1, which is known to be a marker for regulatory T cells in DR-BB rats, and found that 86.9 ± 5.8% of this population expresses RT6.1 (Y.-H.C., H.S.J., and J.-W.Y., unpublished data). This result indicates that CD45RC⁺CD4⁺ T cells may be composed of heterogenous phenotypes.

DR-BB rats show thymic epithelial defects similar to that in DP-BB rats, indicating that DR-BB rats fail to deplete autoreactive T cells (54). Further studies showed that the thymocytes of DR-BB rats have autoreactive potential and can induce diabetes in athymic recipients, providing evidence for a defect in thymic selection in these rats (55). As a result, DR-BB rats may contain autoreactive T cells in the periphery, but these autoreactive T cells may be suppressed by a regulatory T cell subset, such as Th2-like CD45RC⁺CD4⁺ CD8⁻ T cells. This preferential up-regulation of CD45RC⁺ marker for regulatory T cells in DR-BB rats, and found that 5.8% of this population expresses RT6.1 (Y.-H.C., H.S.J., and J.-W.Y., unpublished data). This result indicates that the activated Th1-like cells may be energized in response to the secondary immunization, because Th1-type T cells are more susceptible to anergy and activation-induced cell death, whereas Th2-type T cells are more resistant (49–53).

In conclusion, KRV-induced autoimmune diabetes in DR-BB rats is unlikely to be due to molecular mimicry such as a common epitope between a KRV-specific peptide and a β cell autoantigen. In contrast, KRV infection of DR-BB rats results in the up-regulation of β cell-specific cytotoxic CD8⁺ T cells and Th1-like CD45RC⁺CD4⁺ T cells and the down-regulation of Th2-like CD45RC⁺CD4⁺ T cells. This preferential up-regulation of CD45RC⁺CD4⁺ and CD8⁺ T cells results in the breakdown of the finely tuned immune balance, leading to KRV-induced autoimmune diabetes in DR-BB rats.

**Acknowledgments**

We thank Dr. P. Tattersall, who generously provided rabbit polyclonal Ab against the nonstructural proteins NS1 and NS2 of the minute virus of mice, and Dr. B. Moss, who provided the vaccinia virus vector, pGS20. We
also thank K. Hirasa for insulin scoring, L. Bryant for assistance with flow cytometry, and Dr. A. L. Kyle and K. Clarke for editorial assistance.

References

nopathogenesis of diabetes mellitus. Diabetes Rev. 1:45.
clopedia of Immunology, 2nd Ed. I. M. Rott and P. J. Delves, eds. Academic 
Spontaneous diabetes mellitus: reversal and prevention in the BB/W rat with 
antiserum to rat lymphocytes. Science 206:1421.
betis in rats: destruction of islets is prevented by immunological tolerance. Sci- 
ence 213:1799.
ocythemia and diabetes in insulin-deficient BB rats by the depletion of macrophages. Diabetologia 31:400.
β-cell destruction. V. Decrease of macrophage-dependent T-lymphocytes and NK 
Kilham’s rat virus in diabetes-resistant BB/Wor Rats. Science 254:1010.
J. W. Yoon. 1997. Role of macrophages and macrophage-derived cytokines in the 
pathogenesis of Kilham rat virus-induced autoimmune diabetes-resistant Bio-
nemonic and dimeric forms of replicative intermediates of Kilham rat virus DNA. J. Virol. 20:86.
β-cell destruction. V. Decrease of macrophage-dependent T-lymphocytes and NK 