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Neither B Lymphocytes Nor Antibodies Directed Against Self Antigens of the Islets of Langerhans Are Required for Development of Virus-Induced Autoimmune Diabetes1,2

Andreas Holz,3,* Thomas Dyrberg,† William Hagopian,‡ Dirk Homann,§ Matthias von Herrath,* and Michael B. A. Oldstone*

We evaluated the role of the humoral arm of the immune response in causing or contributing to virus-induced diabetes. Transgenic mice expressing the nucleoprotein (NP) or glycoprotein (GP) of the lymphocytic choriomeningitis virus (LCMV) under control of the rat insulin promoter (RIP) in pancreatic β cells (RIP-LCMV) and RIP-LCMV mice with genetic dysfunction of B cells (RIP-LCMV × μMT/μMT) were compared for development of diabetes after challenge with LCMV. After inoculation with LCMV, B and T lymphocytes and macrophages infiltrated into pancreatic islets in RIP-LCMV mice, and over 50% of these mice generated Abs against host insulin or glutamate decarboxylase. However, neither B cells nor the autoantibodies played a direct role in the initiation, kinetics, or severity of the virus-induced diabetes as judged by comparing disease in RIP-LCMV mice to littermates whose functional B cells were genetically eliminated. Furthermore, the quality and quantity of T lymphocyte and macrophage infiltration was similar in the B cell-deficient and non-B cell-deficient RIP-LCMV mice. Although the development of autoantibodies to islet Ags had no direct influence on the pathogenesis of insulin-dependent (type 1) diabetes mellitus, it served as a prediabetes marker, as such autoantibodies were often elevated before the onset of disease. Hence, the RIP-LCMV model is not only useful for understanding the pathogenetic mechanisms of how islets are destroyed and spared but also for evaluating therapeutic strategies before onset of clinical diabetes. The Journal of Immunology, 2000, 165: 5945–5953.

Insulin-dependent diabetes mellitus (type I; IDDM) is characterized by infiltration of mononuclear cells into the pancreatic islets of Langerhans and the subsequent destruction of insulin-producing β cells (1). The infiltrate is composed primarily of T lymphocytes; however, B lymphocytes and macrophages also are present (2, 3). Although the contributions of T lymphocytes to autoimmune diabetes are appreciated, the significance of B lymphocyte involvement is less well understood and controversial (4–10). B lymphocytes could contribute to disease pathogenesis by producing autoantibodies that cause islet cell damage or by acting as APCs in the local milieu where the disease occurs. Most patients with autoimmune diabetes generate autoantibodies specific to islet-Ags (11–14). These islet-specific autoantibodies serve as important diagnostic markers (15) and may additionally contribute to the disease process through mechanisms of Ab-induced immune pathologic injury (16–18). B cells might also act as APCs during the autoimmune process because they have high affinity cell surface Ag receptors (Igs) and constitutively express MHC molecules. In this scenario, B lymphocytes could present captured self-Ags within the target organ undergoing autoimmune destruction, thereby potentiating the local (auto)immune response (19).

We evaluated the role of B lymphocytes in autoimmune diabetes using the rat insulin promoter (RIP) lymphocytic choriomeningitis virus (LCMV) transgenic mouse model for type 1 diabetes (8, 9, 20). RIP-LCMV mice express either the glycoprotein (GP; RIP-GP transgenic lines) or the nucleoprotein (NP; RIP-NP transgenic lines) of LCMV under control of the RIP. These transgenic mice do not spontaneously develop either lymphocytic infiltration into their pancreas or autoimmune diabetes. However, following infection with LCMV, RIP-GP and RIP-NP mice develop an anti-viral immune response leading to mononuclear cell infiltration into the islets and the destruction of β cells with resultant clinical and biochemical (hyperglycemia and hypoinsulinemia) manifestations of diabetes (8, 20). Both lines of RIP-LCMV transgenic mice are dependent on CD8+ T lymphocytes to develop IDDM. Mice expressing the transgene in the thymus in addition to β cells are dependent on the participation of CD4+ T lymphocytes for development of diabetes. Transgenic mice that do not express the transgene in their thymus do not require CD4+ T cells to develop disease (9). Regardless of which transgenic model is used, the mononuclear cell infiltrate detected in diabetic RIP-LCMV mice consists...
of CD8+ and CD4+ T lymphocytes (9), a picture similar to that observed in humans with type 1 diabetes.

We evaluated the role of B lymphocytes and their products in contributing to IDDM and report here that although B lymphocytes infiltrated the pancreatic islets in RIP-LCMV mice after infection with LCMV andAbs against pancreatic β cells Ags were generated, B lymphocytes were not required for disease. This conclusion was reached as we found that RIP-LCMV mice with their B lymphocytes genetically knocked out developed autoimmune diabetes with the same kinetics and intensity as their RIP-LCMV B cell-competent age and sex matched littermates.

Materials and Methods

Generation of animals and screening

Transgenic RIP-GP and RIP-NP animals deficient of B lymphocytes were generated by breeding μMT/μMT (B cell−/−) mice (21) with RIP-LCMV transgenic mouse lines (8, 9). Both the μMT mice (purchased from The Jackson Laboratory, Bar Harbor, ME) and the RIP-NP and RIP-GP mice were bred to C57BL/6J H-2b mice for at least 8 generations before they were used. Offspring of RIP-LCMV × μMT mice were backcrossed for four generations to RIP-LCMV transgenic mice before being used in diabetes studies.

PCR (21, 22) or Southern blotting (9) determined the genotype of transgenic animals. Two independent standard PCRs were performed to test for the presence of the wild-type μ-chain gene and the disrupted copy of this locus (μMT) (21). The PCR primer pair used to detect the wild-type μ-chain gene was IgM-sense (5'-CTCTGGTGAGTCACCACC) and IgM-antisense (5'-CTTCTCCTCCACGTACCACC). The disrupted μ-chain gene (μMT) (21) was detected by the PCR primer combination neomycin-sense (5'-CCGCGCACAGTCGATGAATCC) and IgM-antisense. The presence of the RIP-GP and RIP-NP transgenes was determined either by Southern blotting as described (9) or by standard PCR (22). For detection of the RIP-GP transgene by PCR, the primer pair GP-sense (5'-GGACAGGCTCAGATGGCAAGA) and GP-antisense (5'-CTCAAAGCAGCCTTGTTGTAGTC) was used. To test for the presence of the RIP-NP transgene, the primers NP-sense (5'-CAGTTATAGGTGCTCTTCGGC) and NP-antisense (5'-AGATCTGGAGGCCTTGCTTTTC) were used.

**FIGURE 1.** B lymphocytes infiltrate the islets of Langerhans. Detection of B lymphocytes (first row) and mouse IgG1 Ig (second row) in the pancreas of diabetic RIP-GP (left) and RIP-NP (right) mice. The third row shows diminished staining for insulin in the infiltrated islets. The bottom row demonstrates the absence of staining for B lymphocytes and Ig in the infiltrated pancreatic islets of RIP-NP mice deficient for B cells. The observed infiltration of B lymphocytes was restricted to the islets of Langerhans. The pancreata of RIP-GP and RIP-NP mice were analyzed 14 and 45 days after infection with LCMV. Infiltration of B cells into the pancreatic islets was absent in non-transgenic littermates infected with LCMV (data not shown), and incubation of pancreatic tissue sections with an anti-rat (secondary) Ab did not cause any staining (data not shown). B cells were detected by immunohistochemical staining with a rat Ab to B220. The presence of Igs in the islets was visualized using an Ab to mouse IgG1. All sections shown were counterstained with hematoxylin. See Materials and Methods for details.
Induction of IDDM in transgenic animals and blood glucose measurements

IDDM was induced in age-matched (5- to 6-wk-old) transgenic RIP-LCMV \( \times \mu MT \) mouse lines (H-2\( ^b \)) by a single i.p. injection of \( 10^5 \) PFU LCMV strain Armstrong (clone 53b) (8). Virus used for IDDM induction was plaque purified three times on Vero cells, and viral stocks were prepared by a single passage on BHK-21 cells as described (8).

After inoculation with virus, mice were checked weekly for diabetes by determining their blood glucose concentration. Animals were considered diabetic when glucose values were \( \geq 300 \) mg/dl. Blood samples were obtained from the retro-orbital plexus of mice, and their glucose concentrations assessed with the ACCUCHECK III system (Boehringer Mannheim, Indianapolis, IN) (9).

Detection of autoantibodies specific to glutamate decarboxylase (GAD), ICA512/IA2, and insulin

Serum autoantibodies in RIP-LCMV mice were measured using radiobinding assays similar to those for detection of autoantibodies in human type 1 diabetes (23, 24). Mouse recombinant GAD65 (whole molecule), human recombinant GAD67 (whole molecule), human recombinant ICA512/IA2 (cytoplasmic domain), and human recombinant insulin (IAA) were used as Ags in the radiobinding assays. The human Ags used displayed a high
homology to the corresponding mouse protein. The Ag insulin was purchased radiolabeled with 125I as mono-iodoTyr-A14-insulin. GAD65, GAD67, and ICA512/IA2 were radiolabeled using 35S-Met as described (25). The complex of Ag containing 30,000 cpm/sample and Ab (4 μl of mouse serum) was allowed to form for 24 h (GAD65, GAD67, ICA512/IA2) or 72 h (IAA). Ag-Ab complexes were precipitated with 10% TCA. Samples were run in duplicate. Mouse Ig-Ag complex was precipitated with Protein G-Sepharose (Zymed, San Francisco, CA). Pellets were washed 5 times, and the radioactivity was counted. Sera containing known amounts of autoantibodies were run concurrently as positive controls as well as negative controls from pooled wild-type (nontransgenic) mouse sera. All assays had signal-to-noise ratio (positive control cpm/negative control cpm) > 20. Indices for insulin autoantibodies were calculated as described (24).

Histology and immunohistochemistry

Tissue for histologic evaluation was fixed in 10% Bouin’s solution, and paraffin-embedded tissue sections were stained with hematoxylin and eosin. Immunohistochemical studies were conducted on 8-μm cryomicrotome sections fixed in ice-cold ethanol (95%) as described (22). Briefly, tissue sections were incubated in 2% FBS and PBS for 1 h and treated with an avidin-biotin blocking kit (Vector Laboratories, Burlingame, CA) to avoid nonspecific staining. T cells, B cells, and macrophages were detected with cell type-specific rat mAbs (anti-mouse-CD4 (L3T4), anti-mouse-CD8 (Ly-2 and Ly-3.2), anti-mouse-CD19 (BD3), and anti-mouse-B220 (RA3-6B2)) and murine CD45R (RA3-6B2) (all obtained from PharMingen, San Diego, CA), and anti-mouse-F4/80 (C1;3A-1, Serotec, Raleigh, NC). Staining for insulin was performed with a guinea pig anti-mouse insulin Ab (Dako, Carpinteria, CA). For Ab detection, sections were incubated with a biotinylated rat anti-mouse IgG Ab (Boehringer Mannheim). After incubation with an anti-rat or anti-guinea pig biotinylated secondary Ab (Vector Laboratories) and a streptavidin-HRP complex (Vector Laboratories, Burlingame, CA), sections were stained with diaminobenzidine (DAB; Zymed). Counterstaining was performed with hematoxylin (Sigma, St. Louis, MO). Slides were embedded in Aquamount (Fisher, Pittsburgh, PA) before being photographed.

CTL assays

Primary CTL activity of splenocytic lymphocytes obtained from RIP-LCMV × μMT/μMT (H-2b) or RIP-LCMV (H-2b) mice was determined 7 days after infection with LCMV. CTL activity was measured using an in vitro 51Cr release assay (22). In brief, syngeneic (MC57; H-2b) and allogeneic (BALB/c; H-2d) target cells were infected either with LCMV strain Armstrong (multiplicity of infection = 1) or with a recombinant vaccinia virus expressing LCMV-NP (8). After 48 h, infected target cells were labeled with 31Cr (Amersham, Arlington Heights, IL) for 1 h, washed with medium, and transferred to 96-well dishes (104 cells per well). In other assays, uninfected target cells were similarly labeled with 31Cr and compared with 10 μg/ml MHC class I Dβ-restricted LCMV peptides GP aa 33–41 and NP aa 396–404. The ratios of splenic effector cells to 31Cr-labeled target cells were 50:1, 25:1, 12.5:1, and 6:1. Target cells, which were incubated with media only or with 1% Nonidet P-40, defined the spontaneous release background and total 51Cr release, respectively. After a 5 1/2 hour incubation of target with effector cells, culture supernatants were centrifuged. Tissue samples and sera were obtained from four to five individual animals per group at 5, 14, and 65 days after infection with 106 PFU LCMV i.p. Viral titers were determined using log dilutions of materials plaque'd on Vero cells (22).

Results

B lymphocytes and their products are present in pancreatic infiltrates of diabetic RIP-LCMV mice

We demonstrated the presence of B lymphocytes and their products in the mononuclear islet infiltrates in virally infected RIP-NP and RIP-GP mice (Fig. 1). In contrast, nontransgenic littermates similarly infected with the virus had no such infiltration in the restricted GP aa 33–41 or NP aa 396–404 or the MHC class II (I-Aβ)-restricted GP aa 61–80 or NP aa 309–328. After surface staining with anti-CD4 (L3T4, CyChrome-conjugated) and anti-CD8 (Ly-2, APC conjugated), intracellular IFN-γ was detected using a murine-specific Ab (XMG1.2, PE-conjugated) (22, 26).

Determination of viral titers

Spleen and kidney tissues were weighed, homogenized in medium to make a 10% solution, and cleared of tissue debris by low speed (1500 × g) centrifugation. Tissue samples and sera were obtained from four to five individual animals per group at 5, 14, and 65 days after infection with 106 PFU LCMV i.p. Viral titers were determined using log dilutions of materials plaque'd on Vero cells (22).
insulin RIP-LCMV mice generate Abs against the islet-Ags GAD and not complete absence of staining for insulin (Fig. 1). Compared
6
labeled, syngeneic target cells (MC57 fibroblasts) previously infected with LCMV.
RIP-GP and RIP-NP mice either deficient (B cell

inductive and sensitive indicator. Fig. 2
erologous GAD65 were elevated in some mice but was a less re-

Table II. Anti-LCMV (self) CTL responses in RIP-NP × μMT and nontransgenic littermatesa

<table>
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<tr>
<th>Mouse Genotype</th>
<th>Specific Lysis (%) of Syngeneic (H-2b) Target Cells, LCMV Infected</th>
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<td>E:T</td>
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<td>RIP-GP × B cell−/−</td>
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* Primary anti-LCMV CTL responses of splenocytes obtained from RIP-GP × μMT mice (I) and RIP-NP × μMT mice (II) were documented by 35Cl release assay. RIP-GP and RIP-NP mice either deficient (B cell

IDDM occurs in RIP-LCMV mice in the absence of B lymphocytes and their products

The presence of B lymphocytes and their products (e.g., Abs) in the pancreatic islets of RIP-LCMV mice after infection with LCMV (Fig. 1) and the generation of Abs to islets Ags (Fig. 2) raised the possibility that B cells, in concert with T cells, might participate in the pathogenesis of type 1 diabetes in RIP-LCMV mice. To directly test this possibility, we bred B cell-deficient (μMT/μMT) mice (21) with RIP-GP and RIP-NP transgenic mice. B cell-deficient mice are homozygous for the μMT mutation that results from the targeted disruption of a membrane domain exon of the Ig μ-chain gene (21). Because these homozygous μMT/μMT (B cell−/−) animals lack mature B lymphocytes, they cannot produce Ig. Double transgenic μMT/μMT mice expressing the LCMV transgenes had a disruption of the membrane exon of the μ-chain gene and no B lymphocytes but normal distributions of T cell subsets according to flow cytometric analysis. No Ig was present in sera of RIP-LCMV × B cell−/− mice tested by ELISA. However, RIP-LCMV mice heterozygous for the μMT mutation (B cell+/−) were indistinguishable from B cell−/− wild-type mice in their relative and absolute quantities of splenic B and T lymphocytes and displayed normal and equivalent levels of serum Ig.

Progeny mice obtained by backcrossing RIP-LCMV × μMT mice to RIP-LCMV lines for four generations were used for diabetes studies. Fig. 3A demonstrates that all experimental groups of RIP-GP × μMT mice, including those with no B cells (RIP-GP × B cell+/−, +/−, −/−), developed diabetes after infection with LCMV; the incidence and kinetics of disease were similar. Thus, absence of B lymphocytes had no significant effect on the incidence of CD4-independent IDDM. We then analyzed the effect of an absence of B lymphocytes in the RIP-NP transgenic model. In contrast to RIP-GP mice, RIP-NP mice require the participation of both CD8+ and CD4+ T lymphocytes to develop autoimmune diabetes. In addition, RIP-NP mice develop disease more slowly because the majority of their anti-viral (self) high-affinity CTLs are deleted in the thymus due to thymic expression of the viral (NP) self-Ag (9). As in RIP-GP × μMT mice, all experimental groups of RIP-NP × μMT mice (RIP-NP × B cell+/−, +/−, and −/−)
developed diabetes (Fig. 3B). No significant differences in disease incidence or kinetics appeared during comparison of RIP-NP/B cell-competent groups with RIP-NP/B cell-deficient mice.

Anti-LCMV CTL responses that terminate acute LCMV infection and cause IDDM are generated in RIP-LCMV × B cell−/− mice

In the virus-induced RIP-LCMV model of diabetes, overt disease is dependent on the generation of anti-viral (self) CTL (8, 9, 20). Therefore, we determined the generation and effectiveness of the primary anti-LCMV CTL responses generated in RIP-LCMV × μMT mice 7 days after a primary LCMV infection. RIP-GP × B cell−/− as well as RIP-NP × B cell−/− mice produced vigorous anti-LCMV CTL responses of a quantity and quality that were indistinguishable from those of their B-cell-competent littermates as judged by in vitro 51Cr lysis assay (Table I). All groups also eliminated infectious virus from serum, spleen, and kidney by 14 days after LCMV challenge (data not shown). As expected, due to the expression of the NP transgene in the thymus of RIP-NP transgenic mice, CTL responses in RIP-NP × B cell mice were weaker than those found in their nontransgenic littermates who, in contrast,
had high affinity LCMV CTL (Table II). These observations were independent of the presence or absence of B lymphocytes.

We assessed the ability of CD8\(^+\) T lymphocytes recovered from the spleens of RIP-NP \(\times\) B cell\(^{-/-}\) mouse (left) and a RIP-NP \(\times\) B cell\(^{+/+}\) mouse (right). Sections shown were obtained from diabetic animals 45–50 days after infection with 10\(^5\) PFU LCMV i.p. Neither differences in the distribution and amount of infiltrating mononuclear cells nor those in the CD8/CD4 T cell ratios were observed in B cell-competent vs B cell-deficient RIP-NP mice. This was also the case in the 4–5 additional mice analyzed for each experimental group. After DAB staining visualized CD4\(^+\) and CD8\(^+\) T cells and macrophages, tissue sections were counterstained with hematoxylin. A corresponding B lymphocyte stain using the B220 Ab confirmed the absence of B cells in the spleen and pancreas of RIP-NP \(\times\) B cell\(^{-/-}\) mice (data not shown).

**FIGURE 6.** Localization of mononuclear cell types in RIP-NP mice deficient or competent in B lymphocytes. Infiltration of CD8\(^+\) T lymphocytes (*top*), CD4\(^+\) T lymphocytes (*middle*), and macrophages (*Mφ; bottom*) into the pancreas of a RIP-NP \(\times\) B cell\(^{+/+}\) mouse (left) and a RIP-NP \(\times\) B cell\(^{-/-}\) mouse (right). Sections shown were obtained from diabetic animals 45–50 days after infection with 10\(^5\) PFU LCMV i.p. Neither differences in the distribution and amount of infiltrating mononuclear cells nor those in the CD8/CD4 T cell ratios were observed in B cell-competent vs B cell-deficient RIP-NP mice. This was also the case in the 4–5 additional mice analyzed for each experimental group. After DAB staining visualized CD4\(^+\) and CD8\(^+\) T cells and macrophages, tissue sections were counterstained with hematoxylin. A corresponding B lymphocyte stain using the B220 Ab confirmed the absence of B cells in the spleen and pancreas of RIP-NP \(\times\) B cell\(^{-/-}\) mice (data not shown).

RIP-NP mice compared with nontransgenic littermates, it is likely that these CTL were of lower affinity as reported elsewhere (9).

**CD8\(^+\) and CD4\(^+\) T cell and macrophage infiltration in the islets of Langerhans is equivalent in RIP-GP and RIP-NP mice whether they are B cell competent or deficient**

Finally, we used immunohistochemistry to analyze the profile of mononuclear cells entering the pancreata of RIP-LCMV \(\times\) B cell\(^{+/+}\) and \(^{-/-}\) mice (Figs. 5 and 6). After infection with LCMV, CD8\(^+\) as well as CD4\(^+\) T lymphocytes infiltrated the pancreas and specifically homed to the islets of RIP-GP animals either deficient or competent in B lymphocytes were equal and indistinguishable from one other. Corresponding findings were obtained when macrophages infiltrating the pancreas were identified by using F4/80 Ab (Fig. 5). Thus, the proportions of individual mononuclear cell subsets (B lymphocytes excluded) remained similar independently of the presence or absence of B lymphocytes (see Fig. 5). Results were comparable for CD8\(^+\) and CD4\(^+\) T lymphocytes and macrophages in the pancreatic islets of RIP-NP \(\times\) B cell\(^{-/-}\) mice vs their B cell\(^{+/+}\) littermates (Fig. 6).

**Discussion**

This paper documents that the absence of B lymphocytes or their products does not affect the incidence or severity of virally induced
type 1 diabetes in the RIP-LCMV transgenic model. Two aspects of this outcome warrant emphasis. First, during the course of diabetes, RIP-LCMV mice developed Abs to islet-specific Ags reflected by the generation of islet cell Abs to insulin and GAD often preceding overt diabetes. Because histological as well as clinical evidence for diabetes occurred in the absence of B lymphocytes and their Ab products in RIP-LCMV mouse lines, by analogy anti-islet cell Abs were not required for disease development. However, these islet Abs can be useful markers for detecting a prediabetic condition in the mouse as they are in humans (15, 32). The incidence of Abs specific for the GAD isoforms and insulin found in RIP-LCMV mice resembled that reported in studies of humans (12, 23, 24, 27, 28). It is unclear how these autoantibodies are generated, but they may reflect epitope spreading where islet destruction releases cryptic self-Ags that can then be presented to immune cells (33). We are currently evaluating the pathogenetic role of epitope spreading in the RIP-LCMV mice. As with the RIP-LCMV model (Fig. 2), Abs to insulin and GAD appear in the nonobese diabetic (NOD) mouse (7, 34). In agreement with studies in NOD mice (35), we were unable to detect Abs to ICA512/IA2 in the RIP-LCMV mice despite their ready identification in humans (29, 30, 36). Second, diabetes occurred in B cell-deficient RIP-LCMV mice, and IDDM occurred whether or not the viral transgene was expressed in the thymus.

It was previously noted that B cell-deficient \( \mu MT/\mu MT \) mice upon challenge with LCMV were capable of generating a LCMV-specific CTL response leading to elimination of the infecting virus similar to clearance of virus in B lymphocyte-sufficient mice (26, 31). This study extends these observations in B cell-deficient RIP-LCMV mice. The frequencies of virus-specific CTL were comparable in wild-type, B cell \(-/-\), RIP-LCMV \( \times \) B cell \(-/-\), and RIP-LCMV \( \times \) B cell \(+/+\) mice (Table I). However, B cell-deficient \( \mu MT/\mu MT \) mice have significantly smaller spleens, resulting in reduced total numbers of lymphocytes reflecting decreased absolute numbers of virus-specific CTLs per spleen.

\( \mu MT/\mu MT \) mice have a partial defect in CD4 \( ^+ \) T cell helper function (26). How can this CD4 \( ^+ \) T cell defect be reconciled with equivalent kinetics in the incidence of diabetes in RIP-LCMV \( \times \) B cell \(-/-\) and B cell \(+/+\) mice that also express the LCMV transgene in their thymus and require CD4 \( ^+ \) T cell help along with effecter CD8 \( ^+ \) CTL (Fig. 3)? An explanation for this observation likely lies in the amounts of virus-specific CD8 \( ^+ \) and CD4 \( ^+ \) T lymphocytes required to cause autoimmune diabetes in the CD4 \( ^+ \) T cell-dependent RIP-LCMV model. Earlier we reported that one or more LCMV-specific CTL per 1000 splenic CD8 \( ^+ \) T cell CTL (\( \sim 100,000 \) CTLs per spleen) are required to cause diabetes in the RIP-LCMV model (37). Accordingly, protection from IDDM was observed when \( \sim 10,000 \) (or fewer) virus-specific CTLs were present per spleen (22, 38). In other studies we found that a cell ratio of \( \sim 10:1 \) of virus-specific CD8 \( ^+ \) CTLs to virus-specific CD4 \( ^+ \) T lymphocytes was required to control a persistent LCMV Arm infection (39). Although the exact number of CD4 \( ^+ \) T lymphocytes required to cause diabetes in the CD4 \( ^+ \) T cell-dependent RIP-LCMV mice is not known, it is likely that fewer CD4 \( ^+ \) T cells than CD8 \( ^+ \) T cells are needed for IDDM induction. Because \( \sim 100,000 \) virus-specific CD8 \( ^+ \) CTLs are necessary for IDDM induction in the RIP-LCMV model by analogy, presumably \( \sim 10,000 \) virus-specific CD4 \( ^+ \) T helper cells would be required in the CD4\( ^+\)-dependent RIP-LCMV transgenics. The numbers of virus-specific CD4 \( ^+ \) T cells generated by RIP-LCMV \( \times \) \( \mu MT/\mu MT \) mice (68,200 \( \pm \) 10,300 LCMV-specific CD4 \( ^+ \) T cells; see Fig. 4) are well above this required CD4 \( ^+ \) T lymphocyte threshold.

A role for the participation of B lymphocytes in the spontaneous IDDM of NOD mice has been reported (40–45). Why does the absence of B lymphocytes affect diabetes in NOD but not RIP-LCMV mice despite the fact that both models require the participation of CD8\( ^+ \) as well as CD4\( ^+ \) T lymphocytes? Likely, different quantitative amounts of CD8\( ^+ \) and CD4\( ^+ \) T cells for the development of IDDM are required for the two models. Other differences may be in the Abs and APCs necessary to initiate IDDM (44–47).

A major advantage of the RIP-LCMV model for the study of IDDM is that the initiating factor, i.e., LCMV infection, is known, and the kinetics of disease can be timed from an exact initiating event throughout its course. The specific cause of the IDDM has been established as the immune response against LCMV because the disease can be totally aborted by peptide therapy that specifically blocks the numbers of available LCMV Ag-specific CD8 \( ^+ \) T lymphocytes (38). Furthermore, the characterization and biology of LCMV-specific CD8\( ^+ \) and CD4\( ^+ \) T cells and their subsets are well known (48). Although the immune response against islet Ags does not appear to play a role in the development of IDDM in the RIP-LCMV model, it does serve as a marker reflecting islet damage. With RIP-LCMV mice, similar to type 1 diabetes in humans, the vast majority of islets producing insulin must be destroyed before concomitant blood glucose levels rise to signal islet disease. The formation of Abs to islet Ags early in the clinical course before onset of IDDM as judged by elevated blood glucose levels allows introduction of therapies to block ongoing disease progression before sufficient islet destruction causes disease. We currently design small peptide molecules as well as organic compounds that mimic such peptides as potential reagents for treatment of preclinical IDDM.

References


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