Interferon-γ Is Essential for Destruction of β Cells and Development of Insulin-dependent Diabetes Mellitus

By Matthias G. von Herrath and Michael B.A. Oldstone

From The Scripps Research Institute, Division of Virology, Department of Neuropharmacology, La Jolla, California 92037

Summary

Autoimmune mediated destruction of β cells of the islets of Langerhans leads to insulin-dependent diabetes mellitus (IDDM). Rat insulin promoter (RIP) lymphocytic choriomeningitis virus (LCMV) transgenic mice that express the nucleoprotein (NP) or glycoprotein (GP) of LCMV under control of the RIP in their β cells develop IDDM after infection with LCMV and serve as a model for virus-induced IDDM. Recently, Kagi et al. (Kagi, D., B. Odermatt, P. Ohashi, R.M. Zinkernagel, and H. Hengartner. 1996. J. Exp. Med. 183:2143–2149) showed, using RIP LCMV perforin-deficient mice, that IDDM does not occur in the absence of perforin. They concluded that perforin-mediated killing by cytotoxic T lymphocytes (CTLs) is the main factor needed for β cell injury and destruction. Here we provide evidence that killing of β cells is more complex and multifactorial. By the use of our RIP LCMV model, we show that in perforin competent but interferon-γ (IFN-γ)-deficient mice, β cell injury is limited and IDDM does not occur. For these studies, double transgenic mice were generated that were genetically deficient in the production of IFN-γ and express LCMV NP or GP in their β cells. In such mice, IDDM was aborted despite the generation of LCMV-specific antiself CTLs that displayed normal cytolytic activity in vitro and in vivo and entered the pancreas. However, mononuclear infiltration into the islets did not occur, and upregulation of class I and II molecules usually found in islets of RIP LCMV single transgenic mice after LCMV infection preceding the onset of clinical IDDM was not present in these bigenic mice. Our findings indicate that in addition to perforin, β cell destruction, development of insulitis, and IDDM also depend on the cytokine INF-γ, presumably through enhancement of major histocompatibility complex expression and antigen presentation.

Destruction of the insulin-producing β cells located in the islets of Langerhans leads to insulin-dependent diabetes mellitus (IDDM)1 (1–3). Host genes, T cell autoimmune responses (4), cytokines (5) and viruses (2, 6) have been implicated in the initiation and progression of this disease (8–14). Immune responses including autoimmune reactions are believed to be regulated by a balance of Th1 (inflammatory) and Th2 (regulatory) cytokines (5, 15–18). IFN-γ is a Th1-type cytokine that plays an intricate role in antiviral host defense mechanisms, activation of CTLs, and enhancement of inflammatory reactions (18–24). Expression of IFN-γ under control of the rat insulin promoter (RIP) in transgenic mice leads to MHC upregulation, inflammation, and IDDM (11, 22). When expressed in β cells of transgenic mice together with viral antigen, it leads to spontaneous development of autoimmune diabetes with generation of antiself (viral) CTLs in the absence of viral challenge (12).

We and others have created transgenic (tg) mouse models to dissect the role(s) played by various components of the immune system leading to IDDM (6, 8, 25). tg mice expressing a viral (“self”) nucleoprotein (NP) or glycoprotein (GP) gene of lymphocytic choriomeningitis virus (LCMV) in pancreatic β cells under control of the RIP fail to develop IDDM spontaneously (defined as hyperglycemia, hypoinsulinemia, mononuclear infiltration into and destruction of the islets of Langerhans) even over a 15-mo observation period (6). However, these mice are not tolerant to the transgene. First, peripheral lymphocytes from these transgenic mice can be primed in vitro to generate primary anti-LCMV CTLs after incubation with Drosophila cells expressing MHC class I molecules and the appropriate LCMV peptide (26). Second, upon infection with LCMV, >95% develop IDDM due to the generation of an antiviral (antiself) CD8+ CTL response. Studies including adoptive transfer of CD8+ cells recovered from islets, immunochemical depletion, and use of CD8 knockout mice indicate that

---

1Abbreviations used in this paper: ARM, Armstrong; GP, glycoprotein; IDDM, insulin-dependent diabetes mellitus; LCMV, lymphocytic choriomeningitis virus; MHC, major histocompatibility complex; NOD, nonobese diabetic; NP, nucleoprotein; RIP, rat insulin promoter; tg, transgenic.
these effector CTL are responsible for initiating the process leading to selective and progressive damage of pancreatic β cells and IDDM (6, 25).

RIP LCMV transgenic mice that express the viral transgene in the pancreas and in the thymus delete high affinity, but not low affinity, antiserum CTLs and, as a consequence, develop slow-onset IDDM that depends on both CD4+ and CD8+ lymphocytes (25, 27). In contrast, RIP-LCMV transgenic mice that express the viral antigen only in the pancreas, develop a rapid-onset IDDM (2 wk) that depends solely on the action of antiserum CD8+ CTL.

CTL have been implicated as effector cells in other models of IDDM and in human IDDM. For example, studies with nonobese diabetic (NOD) mice showed that IDDM can be transferred by CD8 with nonobese diabetic (NOD) mice showed that IDDM and in human IDDM. For example, studies solely on the action of antiself CD8 pancreatic islets of IDDM and in human IDDM. For example, studies solely on the action of antiself CD8+-restricted killing of β cells could be the initiating event for IDDM (14), and glutaric acid dehydrogenase and insulin-specific CTLs were isolated from NOD mice (30, 31). CTLs kill target cells by a MHC-restricted mechanism involving the recognition of specific peptides presented to the TCR by MHC class I glycoproteins. Killing is mediated by the release of cytotoxic granules containing perforin (32, 33) or by the perforin-independent FAS pathway (35). Recently, Kagi et al. showed that killing of β-cells by CTLs is dependent on the release of perforin, since RIP LCMV transgenic mice with a disrupted perforin gene were unable to develop IDDM after LCMV infection (34).

We questioned whether cytokines, especially IFN-γ, might also play a role in β cell destruction and IDDM. To explore the role of IFN-γ, we generated tg mice that expressed the LCMV viral (“self”) transgene in β cells in the islets of Langerhans and were either competent or genetically deficient in the production of IFN-γ (RIP LCMV IFN-γ+/- or −/− mice). This allowed us to determine whether β cells could be directly destroyed by antiserum CTLs in the absence of IFN-γ. Here we report that despite the generation of high or low affinity autoreactive CTLs, IDDM did not occur in IFN-γ-deficient RIP LCMV transgenic mice. Antiserum (viral) CTLs trafficked to the pancreas and were found around the islets. However, neither infiltration into the islets nor upregulation of MHC class I or class II molecules occurred. We conclude that IFN-γ is a key factor required for the induction and maintenance of the autoimmune destruction of β cells in the islets of Langerhans that leads to IDDM.

Materials and Methods

Transgenic Mouse Lines. Generation and characterization of RIP LCMV tg mice with rapid (8-14 d)- or slow-onset (1-6 mo) IDDM after LCMV infection has been described (25). For rapid onset we chose the RIP GP 34-20 (H-2d) tg line as a prototype. These mice express the viral transgene in the pancreas, but not in the thymus. For the slow-onset IDDM paradigm, the RIP NP 25-3 (H-2d) tg line was selected. RIP NP 25-3 mice express the viral NP in both the pancreas and the thymus, but do not express it in any other tissues (25).
Table 1. Primary CTL levels found in RIP LCMV IFN-γ-deficient or -competent mice

<table>
<thead>
<tr>
<th>Group</th>
<th>Effector day 7 splenocytes</th>
<th>Specific 51Cr release (%) from targets</th>
<th>H-2d</th>
<th>H-2b</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>E/T</td>
<td>LCMV vv/GP N Ppep log pe</td>
<td>LCMV</td>
<td>vv/GP</td>
</tr>
<tr>
<td>H-2d</td>
<td>50:1</td>
<td>78 ± 6</td>
<td>2 ± 1</td>
<td>39 ± 12</td>
</tr>
<tr>
<td>IFN-γ-competent</td>
<td>25:1</td>
<td>58 ± 9</td>
<td>0</td>
<td>36 ± 6</td>
</tr>
<tr>
<td>H-2d</td>
<td>50:1</td>
<td>55 ± 12</td>
<td>2 ± 1</td>
<td>39 ± 8</td>
</tr>
<tr>
<td>IFN-γ-deficient</td>
<td>25:1</td>
<td>38 ± 11</td>
<td>0</td>
<td>29 ± 3</td>
</tr>
<tr>
<td>RIP-NP, H-2d</td>
<td>50:1</td>
<td>28 ± 7</td>
<td>11 ± 4</td>
<td>20 ± 4</td>
</tr>
<tr>
<td>IFN-γ-competent</td>
<td>25:1</td>
<td>15 ± 4</td>
<td>3 ± 2</td>
<td>15 ± 3</td>
</tr>
<tr>
<td>RIP-NP, H-2d</td>
<td>50:1</td>
<td>22 ± 7</td>
<td>12 ± 5</td>
<td>19 ± 3</td>
</tr>
<tr>
<td>IFN-γ-deficient</td>
<td>25:1</td>
<td>8 ± 4</td>
<td>4 ± 4</td>
<td>11 ± 4</td>
</tr>
<tr>
<td>H-2b</td>
<td>50:1</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>IFN-γ-competent</td>
<td>25:1</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>H-2b</td>
<td>50:1</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>IFN-γ-deficient</td>
<td>25:1</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>RIP GP, H-2b</td>
<td>50:1</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>IFN-γ-competent</td>
<td>25:1</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>RIP GP, H-2b</td>
<td>50:1</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>IFN-γ-deficient</td>
<td>25:1</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

CTL assays were performed as described in the Materials and Methods section. Target cells were BALB/C17 (H-2d) fibroblasts uninfected or infected with LCMV (ARM), or vaccinia viruses expressing the complete LCMV GP (vv/GP). Background lysis of uninfected cells was <5% in all assays and was subtracted from the lysis (51Cr release) values shown. Five mice were tested per group and the mean ± 1 SE is displayed. Primary CTL activity found in spleens was assayed on day 7 after LCMV infection. Affinities of antiviral CTLs (*) were recorded according to the minimal concentration of LCMV-NP peptide (RPQASGVYM) required for lysis of target cells by CTLs. Note that high affinity CTLs found in non-tg mice require 2 logs less peptide for lysing target cells than low-affinity CTLs found in tg mice (27). Further, low affinity CTLs required 10 times less anti-CD8 antibody to inhibit CTL killing by 50% (C50 [uCD8]) than high affinity CTLs from non-tg mice.

Results

RIP LCMV (NP or GP) × IFN-γ-deficient tg mice generate antiviral (virus) CTLs after infection with LCMV. As shown in Table 1, RIP LCMV transgenic mice with a competent or dysfunctional IFN-γ gene generated good levels of H-2b-specific or H-2d-restricted primary CTLs after LCMV infection. Note that, as reported previously (25), RIP NP mice...
who express the viral transgene in the thymus have a specific reduction in their LCMV NP-specific CTL activities due to negative thymic selection of high affinity CTLs. CTL activity, however, is not completely aborted, since low affinity CTLs escape the negative selection process and are found in the periphery (6, 27). Affinity was assessed by using log dilutions of LCMV N P peptide H-2d-restricted aa118-126 (RPQASGVYM) to coat target cells in the CTL assay (Table 1). These low affinity CTLs are able to induce slow-onset IDDM in single tg RIP NP mice (6). RIP GP tg mice used as a model for fast-onset IDDM do not express the transgene in the thymus and generate high affinity CTLs that were backcrossed to the RIP LCMV (IFN-\(\gamma\)) background for five generations (see Materials and Methods section for breeding scheme). This backcross for five generations makes an IDDM-protective effect conferred by genes accidentally linked to the IFN-\(\gamma\) (-/-) mutation statistically unlikely (44). Fig. 1 shows that even after the backcross, the incidence of IDDM in IFN-\(\gamma\) (+/+) RIP LCMV littermates was still reduced. These findings made the influence of an IDDM-protective gene linked to the IFN-\(\gamma\) knockout genotype not likely. To address the possibility that an IFN-\(\gamma\) gene dose related effect influenced the kinetics of IDDM, we compared the levels of IFN-\(\gamma\) produced by lymphocytes from RIP LCMV \(\times\) IFN-\(\gamma\) (+/+), (+/-), and (-/-) littermates. The results indicated that the lower incidence of IDDM observed in RIP LCMV IFN-\(\gamma\) (+/-) littermates is due to a relatively lower amount of IFN-\(\gamma\) production. Levels of IFN-\(\gamma\) detected in the supernatant of splenocytes harvested 7 d after LCMV infection and stimulated for 3 d in the presence of LCMV infection (Table 1).

In the Absence of IFN-\(\gamma\), Virus-induced Diabetes Does Not Occur in RIP LCMV tg Mice. IDDM did not occur in RIP GP or NP 25-3 single tg mice unless they were challenged with LCMV ARM (Fig. 1). Fig. 1 also shows that 2-8 wk after receiving 1 \(\times\) 10^5 PFU of LCMV intraperitoneally, most IFN-\(\gamma\)-competent RIP GP (fast-onset IDDM) or NP (slow-onset IDDM) single tg mice develop IDDM. In contrast, virus-inoculated double tg mice (10 mice/group) that were deficient in IFN-\(\gamma\) production did not develop IDDM over a 3-8-mo observation period. Thus, virus-induced IDDM does not occur in the absence of IFN-\(\gamma\), regardless of the affinity of the generated antiviral CTLs.

Incidence of IDDM was reduced and delayed in RIP LCMV IFN-\(\gamma\) (+/-) mice. To ensure that the lack of IDDM in the absence of IFN-\(\gamma\) was not due to the effect of a potential protective gene linked to the IFN-\(\gamma\) (-/-) background, the experiment was repeated usingtg mice that were backcrossed to the RIP LCMV (IFN-\(\gamma\) +/-) background for five generations (see Materials and Methods section for breeding scheme). This backcross for five generations makes an IDDM-protective effect conferred by
infiltrated into, the islets (Fig. 2 F). Lastly, MHC class I (Fig. 2, H and I) expression was upregulated in inflammatory islet lesions and on β cells of RIP LCMV tg mice (Fig. 2 H). In contrast, upregulation of class I (Fig. 2 I) molecules did not occur in IFN-γ-deficient RIP LCMV mice and β cells remained intact. As shown in Fig. 2, A, D, and G, these events were not observed in non-tg mice infected with LCMV. Further studies showed that expression of MHC class II that is usually elevated in islets of tg mice with IDDM, was not detected in islets of tg mice with IFN-γ (−/−) mice (data not shown).

IFN-γ-deficient RIP LCMV tg mice have less LCMV-specific memory CTLs in the Pancreas than their IFN-γ-competent littermates. Levels of LCMV-specific memory CTLs were quantitated in RIP LCMV IFN-γ-competent or -deficient mice. The results are shown in Table 2. Normal and IFN-γ-deficient mice or RIP LCMV tg IFN-γ-competent or -deficient mice generated nearly equivalent amounts of LCMV-specific memory CTLs in their spleens (precursor frequency average 1/1,500). However, fewer LCMV-specific CTLs were detected in the pancreas of RIP LCMV IFN-γ-deficient mice that had not developed IDDM compared to LCMV-specific CTLs recovered from pancreas of IFN-γ-competent RIP LCMV mice that had developed IDDM (Table 2).

Discussion
The major finding documented here is that injury to β cells mediated by CTLs does not occur in the absence of IFN-γ (Fig. 1). Further, IFN-γ is not required for the generation of LCMV-specific CTLs (Table 1) and killing of
target cells in vitro, and clearance of acute LCMV infection in vivo can occur in the absence of IFN-\(\gamma\) (21). Thus, CTLs generated in IFN-\(\gamma\)-deficient mice can lyse target cells in vitro, clear virus infections in vivo, and enter the pancreas in vivo (Fig. 2). A likely mechanism for the ablation of the autoimmune process and the failure of IDDM to occur in the absence of IFN-\(\gamma\) is the insufficient upregulation of MHC class I molecules on \(\beta\) cells and class II molecules on APCs, but not a defect in CTL generation or function. As a consequence, there is a lack of antigen presentation and functional CTLs are not retained in the islets. These findings provide a clear rationale for suppressing inflammatory cytokine levels like IFN-\(\gamma\) locally in the islets as a treatment of IDDM.

IFN-\(\gamma\) is a key cytokine produced by activated CTLs. It is involved in the upregulation of MHC molecules and in antiviral host defense (21, 45–47). Recent data show that IFN-\(\gamma\) knockout mice generate LCMV-specific primary and memory CTLs with equivalent activities as found in non-tg littermates (21). The generation of LCMV-specific primary CTLs in IFN-\(\gamma\)-deficient mice terminates an acute LCMV viral infection (21). However, when memory CTLs from IFN-\(\gamma\)-knockout mice are adoptively transferred into persistently infected recipients, they are unable to clear the virus showing that, while IFN-\(\gamma\) is not required for CTL activity in vitro or control of acute infection in vivo, it is required for viral clearance of a persistent infection by CTLs in vivo (22, 45, 46). The role IFN-\(\gamma\) plays in IDDM is likely mediated by the upregulation of MHC molecules on \(\beta\) cells and APCs in the islets. First, upregulation of MHC class I glycoproteins is a frequent marker during the development of IDDM (11, 26). Second, IDDM does not occur in the absence of MHC class I expression on \(\beta\) cells (25). For example, expression of the adenoviral E3 gene complex can prevent MHC class I trafficking to the cell surface. When the E3 complex is co-expressed with LCMV proteins under the RIP in \(\beta\) cells, upregulation of MHC class I D\(^b\) molecules is suppressed specifically and IDDM is prevented (von Herrath, M., S. Efrat, M. Oldstone, and M. Horwitz, manuscript submitted for publication). However, upregulation of MHC expression itself in the absence of a specific (viral) trigger or in the absence of autoreactive CTLs is not sufficient for the development of IDDM (43). Further, APCs expressing MHC class II are not found in islets of RIP LCMV IFN-\(\gamma\)-deficient or -competent controls. CTL activity was tested on syngeneic LCMV infected or uninfected target cells after a 5–14 d in vitro stimulation (see Materials and Methods), and precursor frequency analysis was performed as described (26). NDT, not detectable (<1/60,000).

<table>
<thead>
<tr>
<th>Group</th>
<th>Origin</th>
<th>Precursor frequency of CTL</th>
<th>Killing of H-2(^d)</th>
<th>H-2(^b)</th>
</tr>
</thead>
<tbody>
<tr>
<td>H-2(^d) IFN-(\gamma)-competent</td>
<td>Spleen</td>
<td>LCMV 1/1,500 NDT 1/2,500 NDT</td>
<td>60 ± 7%</td>
<td></td>
</tr>
<tr>
<td>H-2(^d) IFN-(\gamma)-deficient</td>
<td>Spleen</td>
<td>LCMV 1/3,000 NDT 1/4,200 NDT</td>
<td>54 ± 9%</td>
<td></td>
</tr>
<tr>
<td>RIP NP, H-2(^d) IFN-(\gamma)-competent</td>
<td>Spleen</td>
<td>LCMV 1/3,000 1/9,000 1/3,900 NDT</td>
<td>34 ± 8%</td>
<td></td>
</tr>
<tr>
<td>RIP NP, H-2(^d) IFN-(\gamma)-deficient</td>
<td>Spleen</td>
<td>LCMV 1/5,000 1/9,000 1/7,000 NDT</td>
<td>35 ± 2%</td>
<td></td>
</tr>
<tr>
<td>RIP NP, IDDM IFN-(\gamma)-competent</td>
<td>Pancreas</td>
<td>LCMV 1/2,000 not done 1/6,000 NDT</td>
<td>38 ± 7%</td>
<td></td>
</tr>
<tr>
<td>RIP NP, no IDDM IFN-(\gamma)-deficient</td>
<td>Pancreas</td>
<td>LCMV 1/20,000 NDT NDT NDT</td>
<td>18 ± 5%</td>
<td></td>
</tr>
</tbody>
</table>

Secondary CTLs were recovered from spleens and pancreata of infected (10\(^5\) PFU LCMV intraperitoneally) IFN-\(\gamma\)-deficient RIP LCMV mice, single transgenic RIP LCMV mice, and nontransgenic IFN-\(\gamma\)-deficient or -competent controls. CTL activity was tested on syngeneic LCMV infected or uninfected target cells after a 5–14 d in vitro stimulation (see Materials and Methods), and precursor frequency analysis was performed as described (26). NDT, not detectable (<1/60,000).

Table 2. IFN-\(\gamma\)-deficient RIP LCMV tg Mice Have Fewer LCMV-specific Memory CTLs in the Pancreas than their IFN-\(\gamma\)-competent Littermates.

Recently, Kagi et al. reported that IDDM did not occur in perforin-deficient RIP LCMV transgenic mice and concluded that \(\beta\) cell destruction was predominantly a consequence of perforin-mediated lysis by antiseif (viral) CTLs (34). In this model, virus-induced MHC class I restricted CTLs are the key factor for the induction of IDDM. Diabetes does not occur in the absence of MHC class I expression on \(\beta\) cells (25) or the absence of CD8\(^+\) CTLs (8, 25). Both our own (Tishon, T., and M.B.A. Oldstone, unpublished data) and other results (49) indicate that while perforin-competent CTLs are required to lyse target cells in vitro, they are unable to destroy \(\beta\) cells in vivo in the absence of IFN-\(\gamma\) to cause IDDM (Fig. 1). Thus, the cytokine IFN-\(\gamma\) plays an important role in the pathogenesis of IDDM and...
without it, IDDM does not occur, even after an 8-mo observation period. Kagi et al. (34) reported infiltration and retention of CD8+ lymphocytes in the islets was observed in the absence of perforin although IDDM did not occur over the 2-mo observation period after LCMV infection. Whether IDDM could have occurred at later times in the absence of perforin and in the presence of IFN-γ is unknown.

It has been reported (24) that IDDM is delayed, but not aborted, in NOD mice in the absence of IFN-γ. A likely reason why IDDM occurs in IFN-γ-deficient NOD mice, but fails to occur in the RIP LCMV tg model, is that NOD mice are usually genetically prone to spontaneously develop IDDM (50). They express genes that convey susceptibility to IDDM, and it is likely that their β cells are more sensitive to destruction. By contrast the RIP LCMV tg mice do not spontaneously develop diabetes (6), even after a 2-yr observation period (Tishon, T., and M.B.A. Oldstone, unpublished data).

From our data, it is unlikely that the lack of IDDM in IFN-γ (−−) and the lower incidence of IDDM in IFN-γ (+−) mice is due to an IDDM-protective gene linked to the IFN-γ (−−) mutation for several reasons. First, similar kinetics of IDDM are observed in all groups of mice independent of whether F-1 backcrosses to IFN-γ (−−) background or F-5 backcrosses to the RIP LCMV background or F-5 backcrosses to the RIP LCMV background, and it is proposed that the RIP LCMV background or F-5 backcrosses to the RIP LCMV background are used (Fig. 1). Second, recent work by Krakowsk et al. (51) demonstrated a protective effect of the IFN-γ (−−) genotype for experimental allergic encephalitis (EAE), whereas the encephalitis was enhanced on the IFN-γ (−−) background. Such findings were not mirrored in our report. Finally, and most importantly, we find less IFN-γ production by splenocytes from IFN-γ (+−) compared to IFN-γ (+−) splenocytes. This finding correlates with the lower incidence of IDDM in IFN-γ (+−) mice and suggests a quantitative gene-dosage effect in IFN-γ production as the explanation for the different incidence of IDDM observed in RIP LCMV IFN-γ (+−) and (+−) mice.

In conclusion, a multifactorial process leads to IDDM and autoimmune destruction of β cells. The development of insulinitis involves a cascade of events. Generation of anti-self idet-antigen-specific CTLs, a functional perforin pathway, the presence of IFN-γ, upregulation of MHC class I on β cells, and likely the presence of CD4 lymphocytes are required. Identification of the factors involved will suggest the strategies that can be applied to hinder or prevent the autoimmune process from continuing and hopefully prevent IDDM.

The authors thank Diana Frye for assistance with the manuscript.

This work was supported by United States Public Health Service grant AG04342 to M.B.A. Oldstone and National Institutes of Health grant DK99505 and a Career Development Award of the Juvenile Diabetes Foundation International to Matthias G. von Herrath.

Address correspondence to Dr. Matthias G. von Herrath, Division of Virology, Department of Neuropharmacology, The Scripps Research Institute, 10550 N. Torrey Pines Road, La Jolla, CA 92037.

Received for publication 27 August 1996 and in revised form 14 November 1996.

References


