

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

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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 antiseif 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 insulinitis, 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 de-

velopment of autoimmune diabetes with generation of antiseif (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, hypoin-sulinemia, 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 (antiseif) 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; 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, antiself 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 antiself 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⁺ T cells (28, 29). More recently it has been proposed that specific MHC-restricted killing of β cells could be the initiating event for IDDM (14), and glutamic acid decarboxylase 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 can be directly destroyed by antiself 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. Antiself (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-2^b) 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-2^d) 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).

Mice with a nonfunctional IFN- γ gene were generated by T. Stewart and his colleagues (23) by replacing the normal IFN- γ allele in mouse embryonic stem cells with a defective allele. C57Bl/6 (H-2^b) and BALB/c (H-2^d) IFN- γ -deficient mice were obtained from Genentech Inc. (South San Francisco, CA). Mice homozygous for the mutation in the IFN- γ gene (IFN- γ -deficient or -/- mice) were housed in specific, pathogen-free conditions, were healthy, fertile, had normal life spans, and did not spontaneously develop IDDM.

Double tg mice that express LCMV NP or GP protein in their pancreas and are IFN- γ -deficient were obtained by crossing IFN- γ -deficient mice with RIP LCMV tg mice, and then backcrossing the LCMV-positive littermates from the F1 generation, which are heterozygous for the IFN- γ gene mutation to IFN- γ -deficient mice. As a result, IFN- γ -deficient (-/-) and IFN- γ (+/+) mice were obtained that did or did not express LCMV proteins in their pancreas. These mice were used in experimental and control groups. For further studies, the LCMV positive F1 littermates from the first mating were backcrossed for five generations to the RIP LCMV (IFN- γ +/+) background and the resulting LCMV-positive littermates that were heterozygous for IFN- γ were intercrossed once to generate RIP LCMV IFN- γ -/- and +/-, or +/+ mice for use in experiments. This backcross was necessary to ensure that no IDDM-protective genes were linked to the IFN- γ -/- genetic background.

Biochemical Studies. Transgenic mice were identified by hybridization of DNA extracted from tail biopsies using an LCMV NP- or GP-specific probe (6). RNA was extracted from PBL and organs (thymus, brain, liver, muscle) by the Guanidinium-Isothiocyanate method (25) for analysis. Before PCR analysis, RNA was treated with RNase-free RQ1 DNase to eliminate contaminating the DNA. The reverse transcriptase-PCR was carried out for 40 cycles. LCMV NP-specific primers used were: 5' CAG TTA TAG GTG CTC TTC CGC 3' and 5' AGA TCT GGG AGC CTT GCT TTG 3'. Heterozygosity or homozygosity for the IFN- γ knockout defect was also tested for by PCR. The wild-type IFN- γ gene was detected using primers AGAAGTAAGTG-GAAGGGCCCCAGAAG and AGGGAACTGGGAGAGGAG-AAATAT that resulted in a 220-bp product while the mutant gene was detected with primers TCAGCGCAGGGCGCCCG-GTTCTTT and ATCGACAAGACCGGCTTCCATCCGA that defined a 190-bp product (23, 25).

Virus. The virus used was LCMV Armstrong (ARM), clone 53b. Its origin, quantitation by plaquing, sequence, and biological properties have been described (36–38).

CTL Assay. CTL activity was recorded in a 5–6 h in vitro ⁵¹Cr release assay (6, 25, 39–41). Samples were run in triplicate and variance was <10%. To judge CTL activity, uninfected or infected targets with either LCMV ARM (multiplicity of infection 1), vaccinia virus expressing LCMV NP, or LCMV GP (multiplicity of infection 3) were used. Target cells were H-2^b (MC57) or H-2^d (BALB C17) fibroblasts. Lymphocytes were obtained from spleens of 8–12-wk-old mice either not inoculated or given 2 × 10³ PFU of LCMV ARM intraperitoneally 7 d earlier. Single-cell suspensions were made and used at 50:1, 25:1, and 12:1 effector/target ratios. H-2^b- and H-2^d- restricted LCMV-specific CTL clones (42) were used at effector/target ratios of 10:1, 5:1, and 1:1. Other secondary CTL lines and clones were generated and cloned by limiting dilution (42). For coating with peptides, target cells were preincubated with the respective peptide at 50 μ g/well for 30 min before starting the 4–5 h incubation for the ⁵¹Cr release assay.

Recovery of CTL from Pancreas. CTLs were isolated from the

Table 1. Primary CTL Levels Found in RIP LCMV IFN- γ -deficient or -competent Mice

Effector day 7 splenocytes		Specific ^{51}Cr release (%) from targets					
		H-2 ^d				H-2 ^b	
Group	E/T	LCMV	vv/GP	NPpеп	log pe*	LCMV	vv/GP
H-2 ^d	50:1	78 \pm 6	2 \pm 1	39 \pm 12	-9	0	0
IFN- γ -competent	25:1	58 \pm 9	0	36 \pm 6		0	0
H-2 ^d	50:1	55 \pm 12	2 \pm 1	39 \pm 8	-9	0	0
IFN- γ -deficient	25:1	38 \pm 11	0	29 \pm 3		0	0
RIP-NP, H-2 ^d	50:1	28 \pm 7	11 \pm 4	20 \pm 4	-7	0	0
IFN- γ -competent	25:1	15 \pm 4	3 \pm 2	15 \pm 3		0	0
RIP-NP, H-2 ^d	50:1	22 \pm 7	12 \pm 5	19 \pm 3	-7	0	0
IFN- γ -deficient	25:1	8 \pm 4	4 \pm 4	11 \pm 4		0	0
H-2 ^b	50:1	0	0	0		55 \pm 9	33 \pm 7
IFN- γ -competent	25:1	0	0	0		28 \pm 6	16 \pm 6
H-2 ^b	50:1	0	0	0		60 \pm 9	32 \pm 7
IFN- γ -deficient	25:1	0	0	0		33 \pm 5	20 \pm 2
RIP GP, H-2 ^b	50:1	0	0	0		52 \pm 4	36 \pm 7
IFN- γ -competent	25:1	0	0	0		22 \pm 3	15 \pm 5
RIP GP, H-2 ^b	50:1	0	0	0		60 \pm 6	28 \pm 8
IFN- γ -deficient	25:1	0	0	0		38 \pm 5	22 \pm 8

CTL assays were performed as described in the Materials and Methods section. Target cells were BALB/C17 (H-2^d) 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 (^{51}Cr release) values shown. Five mice were tested per group and the mean \pm 1 SE is displayed. Primary CTL activity found in spleens was assessed on day 7 after LCMV infection. Affinities of antiself 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 [α CD8]) than high affinity CTLs from non-tg mice.

pancreas as described (26). Briefly, pancreatic tissues obtained from tg mice were freed from fat and other tissues, digested with collagenase, and lymphoid cells were recovered by centrifugation through a ficoll-hypaque gradient.

Analysis of IDDM. IDDM was defined by hyperglycemia (blood glucose > 300 mg/dl), low levels of pancreatic insulin (<10 μg of insulin/mg of pancreas), and presence of a mononuclear cell infiltration in the islets. Blood samples were obtained from the retro-orbital plexus of mice at weekly or monthly intervals. Amount of glucose in the blood was determined using Accucheck II strips (Boehringer Mannheim, Indianapolis, IN). Normal blood glucose for age- and sex-matched control was mean \pm 1 SE (20–40 mice/group) 145 \pm 7. Insulin concentration in the pancreas was determined by radioimmuno assay (6) and normal levels of insulin were 40 $\mu\text{g}/\text{mg}$ \pm 12. Absence or presence of mononuclear cells in the islets was studied in tissues fixed in 10% zinc formalin, mounted in paraffin, and stained with hematoxylin and eosin (25).

Immunohistochemical Analyses of Tissues. Tissues were quick frozen in O.C.T. compound, 6–10- μm cryomicrotome sections obtained, fixed for 1 min in 1% paraformaldehyde, washed in PBS, and incubated with avidin/biotin to remove nonspecific binding. Primary antibodies consisting of rat anti-mouse CD4 (clone RM 4-5), anti-CD8 (clone 53-6.7), anti-B220 (clone RA3 6B2), anti-F4/80 (clone A3-1), anti-MAC-1 (clone M 1/70), anti-class I

(clone M 1/42), and anti-class II (clone M5/114), (PharMingen, San Diego, CA and Boehringer Mannheim) were applied for 1 h. After washing, secondary antibody [biotinylated goat anti-rat (or anti-mouse) IgG (Vector Laboratories, Inc., Burlingame, CA)] was added for 1 h. Color reaction was obtained with avidin-peroxidase conjugate (Boehringer Mannheim) and diaminobenzidine in the presence of H₂O₂ (43).

ELISA Assay for IFN- γ . Sandwich ELISA assays for IFN- γ production were carried out as recommended by PharMingen (San Diego, CA), who also provided matched pairs of capture and detection antibodies. Tissue culture supernatants tested in the ELISA were harvested after 3 d in vitro culture of 10⁵ splenocytes obtained 7 d after LCMV infection and cultured in 10% RPMI containing 10⁻⁵ M LCMV NP H-2^d CTL peptide (RPQASGVYM).

Results

RIP LCMV (NP or GP) \times IFN- γ -deficient tg mice generate antiself (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-2^b- or H-2^d-restricted primary CTLs after LCMV infection. Note that, as reported previously (25), RIP NP mice

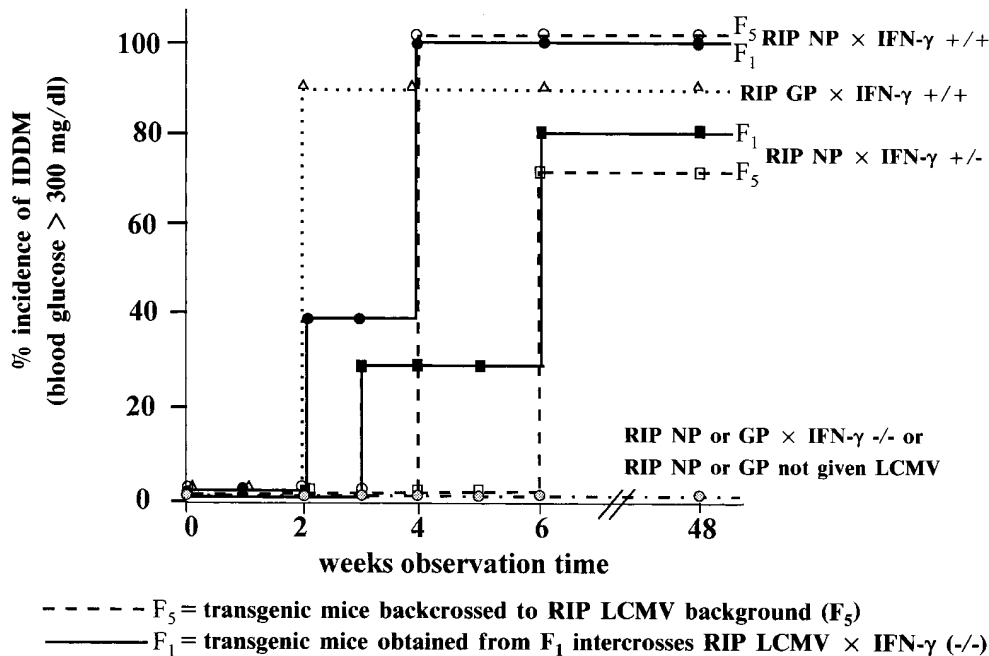


Figure 1. Incidence of IDDM (blood glucose >300 mg/dl) in RIP LCMV (NP or GP) IFN- γ -deficient or -competent tg mice. Blood glucose levels were measured at 2-wk intervals as described in Materials and Methods, 10 mice per group were analyzed. LCMV-treated mice were challenged with 2×10^5 PFU LCMV intraperitoneally.

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 NP peptide H-2^d-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 primary CTLs in the presence and absence of IFN- γ after LCMV infection (Table 1).

In the Absence of IFN- γ , 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 1×10^5 PFU of LCMV intraperitoneally, most IFN- γ -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- γ production did not develop IDDM over a 5-8-mo observation period. Thus, virus induced IDDM does not occur in the absence of IFN- γ , regardless of the affinity of the generated antiself (viral) CTLs.

Incidence of IDDM was reduced and delayed in RIP LCMV IFN- γ (+/-) mice. To ensure that the lack of IDDM in the absence of IFN- γ was not due to the effect of a potential protective gene linked to the IFN- γ (-/-) background, the experiment was repeated using tg mice that were backcrossed to the RIP LCMV (IFN- γ +/+) 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- γ (-/-) mutation statistically unlikely (44). Fig. 1 shows that even after the backcross, the incidence of IDDM in IFN- γ (+/-) RIP LCMV littermates was still reduced. These findings made the influence of an IDDM-protective gene linked to the IFN- γ knockout genotype not likely. To address the possibility that an IFN- γ gene dose related effect influenced the kinetics of IDDM, we compared the levels of IFN- γ produced by lymphocytes from RIP LCMV \times IFN- γ (+/+), (+/-), and (-/-) littermates. The results indicated that the lower incidence of IDDM observed in RIP LCMV IFN- γ (+/-) littermates is due to a relatively lower amount of IFN- γ production. Levels of IFN- γ detected in the supernatant of splenocytes harvested 7 d after LCMV infection and stimulated for 3 d in the presence of LCMV H-2^d (NP) peptide were 2000 ± 400 U/ml for IFN- γ (+/+), 700 ± 320 U/ml for IFN- γ (+/-), and nondetectable for IFN- γ (-/-) littermates.

Immunohistochemical Analysis of Islets from RIP LCMV \times IFN- γ -deficient or Competent tg Mice. Immunohistochemical analysis of islets from RIP LCMV \times IFN- γ -deficient or -competent tg mice was carried out 45 d after infection with LCMV. Results are shown in Fig. 2. In the absence of IFN- γ , insulinitis was markedly reduced (Fig. 2, C versus B). IFN- γ -deficient mice failed to show infiltration into the islets, while islets from IFN- γ -competent RIP LCMV mice showed insulinitis and destruction of most β cells. Further, CD8⁺ CTLs were found in infiltrates of single tg RIP LCMV IFN- γ -competent mice as expected (Fig. 2 E, 25). In contrast, CTL were absent in islets of IFN- γ -deficient mice, but were found in the pancreas or around the islets (Table 2; Fig. 2 J). Thus, despite generation of antiviral CTLs (Table 1) that can enter the pancreas, β cells were not destroyed in IFN- γ -deficient mice. CTLs were seen passing through the pancreas, but were not retained in, or

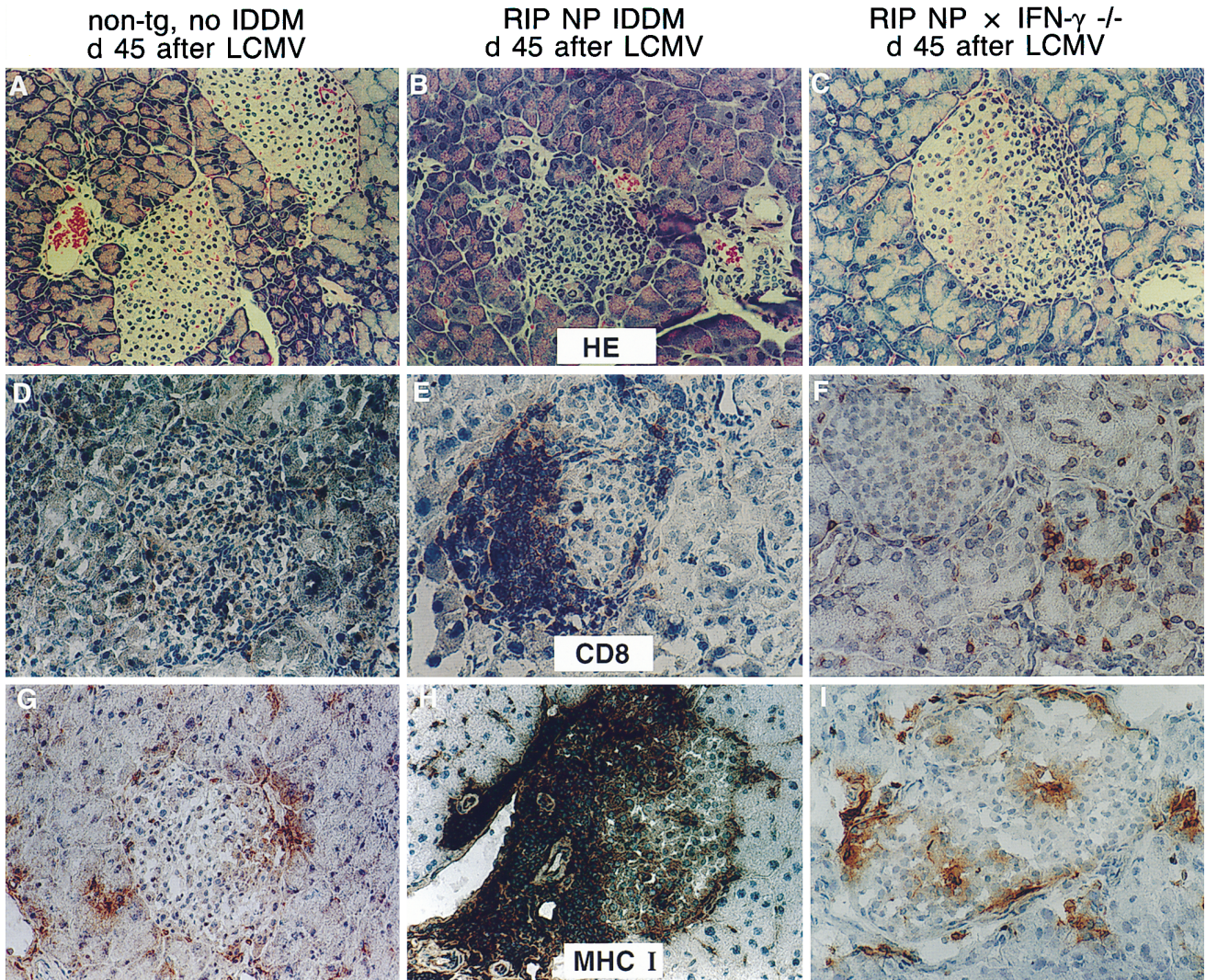


Figure 2. Histopathology and immunochemical analysis of islets of Langerhans in LCMV-infected RIP LCMV (NP) IFN- γ -deficient or -competent tg mice. Immunohistochemical staining of consecutive 5-6 micron sections of islets was carried out as described in Materials and Methods. At least three different areas were surveyed per mouse. 10 mice were studied from each experimental group. Representative sections are shown that were similar for each mouse in that particular group. Sections shown were processed and stained at the same time. Original magnification is 200. Panels A-F indicate tg mouse strain, time after infection, and staining method applied.

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 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

Table 2. *IFN- γ -deficient RIP LCMV tg Mice Have Fewer LCMV-specific Memory CTLs in the Pancreas than their IFN- γ -competent Littermates*

Secondary effector day 60 CTL		Precursor frequency of CTL				Killing of H-2 ^d targets
		H-2 ^d		H-2 ^b		
Group	Origin	LCMV	vv/GP	NPpép	LCMV	LCMV
H-2 ^d IFN- γ -competent	Spleen	1/1,500	NDT	1/2,500	NDT	60 \pm 7%
H-2 ^d IFN- γ -deficient	Spleen	1/3,000	NDT	1/4,200	NDT	54 \pm 9%
RIP NP, H-2 ^d IFN- γ -competent	Spleen	1/3,000	1/9,000	1/3,900	NDT	34 \pm 8%
RIP NP, H-2 ^d IFN- γ -deficient	Spleen	1/5,000	1/9,000	1/7,000	NDT	35 \pm 2%
RIP NP, IDDM IFN- γ -competent	Pancreas	1/2,000	not done	1/6,000	NDT	38 \pm 7%
RIP NP, no IDDM γ -IFN-deficient	Pancreas	1/20,000	NDT	NDT	NDT	18 \pm 5%

Secondary CTLs were recovered from spleens and pancreata of infected (10^3 PFU LCMV intraperitoneally) IFN- γ -deficient RIP LCMV mice, single transgenic RIP LCMV mice, and nontransgenic IFN- γ -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).

target cells in vitro, and clearance of acute LCMV infection in vivo can occur in the absence of IFN- γ (21). Thus, CTLs generated in IFN- γ -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- γ is the insufficient upregulation of MHC class I molecules on β 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- γ locally in the islets as a treatment of IDDM.

IFN- γ 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- γ 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- γ -deficient mice terminates an acute LCMV viral infection (21). However, when memory CTLs from IFN- γ knockout mice are adoptively transferred into persistently infected recipients, they are unable to clear the virus showing that, while IFN- γ 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- γ plays in IDDM is likely mediated by the upregulation of MHC molecules on β 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 β 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 β 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- γ (–/–) mice (data not shown) indicating that the lack of IFN- γ also results in a loss of infiltration by APCs that are required to propagate the autoimmune process leading to IDDM.

IFN- γ could also contribute to the autoimmune process in the pancreas by “bystander” activation of autoreactive CTLs and other inflammatory cells (48). The recovery of lower numbers of antiself (viral) CTLs from the pancreas of IFN- γ -deficient RIP LCMV mice 60 d after infection (Table 2) suggests that this may also occur. Hence, both upregulation of MHC molecules and “bystander” activation may be needed for IDDM to develop.

Recently, Kagi et al. reported that IDDM did not occur in perforin-deficient RIP LCMV transgenic mice and concluded that β cell destruction was predominantly a consequence of perforin-mediated lysis by antiself (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 β 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 β cells in vivo in the absence of IFN- γ to cause IDDM (Fig. 1). Thus, the cytokine IFN- γ 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 are used (Fig. 1). Second, recent work by Krakowski 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 islet-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.

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