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Immunology

Expression of adenoviral E3 transgenes in β cells prevents autoimmune diabetes

(insulin-dependent diabetes mellitus/lymphocytic choriomeningitis virus/major histocompatibility complex expression)

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ABSTRACT **The adenovirus (Ad) genome contains immunoregulatory and cytokine inhibitory genes that are presumed to function in facilitating acute infection or in establishing persistence *in vivo*. Some of these genes are clustered in early region 3 (E3), which contains a 19-kDa glycoprotein (gp19) that inhibits the transport of selected class I major histocompatibility complex (MHC) molecules out of the endoplasmic reticulum. In addition, the E3 region contains three protein inhibitors of the cytolytic function of tumor necrosis factor α (TNF-α). Because type I autoimmune diabetes destroys islets by mechanisms that involve class I MHC and TNF-α, we investigated whether the entire cassette of Ad E3 genes might prevent the onset of diabetes in a well studied lymphocytic choriomeningitis viral (LCMV) murine model of virus-induced autoimmune diabetes. In this model, a LCMV polypeptide (either glycoprotein or nucleoprotein) expressed as a transgene in the islets is a target for autoimmune destruction of β cells after LCMV infection. In this scenario the LCMV-induced immune response is directed not only against the virus but also against the LCMV transgenes expressed in the β cells. Our experiments demonstrated a very efficient prevention of this LCMV-triggered diabetes by the Ad E3 genes. This resulted from the inhibition of target cell recognition by a fully competent and LCMV-primed immune system. Unlike the results from the β-2 microglobulin gene deletion experiments, our approach shows that selective regulation at the level of the target cell is sufficient to prevent autoimmune diabetes without disrupting the function of the systemic immune response. Although the Ad genes in these experiments were provided as transgenes, recent experiments may permit the introduction of such genes through the use of viral vectors. Although the decrease in class I MHC in islets by Ad genes was demonstrated in these *in vivo* studies, the relative importance of this process and the control of TNF-α cytolysis must await further genetic dissection of the introduced Ad genes.**

The adenovirus (Ad) early region 3 (E3) genes encode several proteins that inhibit host immune responses (1, 2). One of them is the Ad type 2 19-kDa glycoprotein (gp19), which binds to class I major histocompatibility complex (MHC) heavy chain and prevents transport of MHC molecules out of the endoplasmic reticulum (3). The class I MHC molecule is known to present viral peptides to cytotoxic T lymphocytes (CTL), which leads to specific recognition and killing of virus-infected target cells (4). The Ad gp19 binds with high affinity to D^b and K^d, but less well to K^b and D^d *in vitro* (2, 3, 5). Studies with transfected cell lines expressing gp19 revealed a decrease in class I MHC-mediated antigen presentation, which impaired subsequent CTL recognition of these cells (3). The Ad E3 gene complex also encodes a 14.7-kDa protein, as well as the heterotrimer of two proteins (10.4 kDa and 14.5 kDa), each of which can inhibit tumor necrosis factor α (TNF-α)-mediated cytolysis of Ad-infected cells (1, 2, 6). Recently, transgenic islets expressing the Ad E3 region genes were shown to survive longer *in vivo* than control islets when transplanted into allogeneic recipients (5).

To analyze the local effects of the Ad E3 gene complex *in vivo* on the protection of a specific target cell such as a β cell from autoimmune destruction, the well characterized rat insulin promoter (RIP)-lymphocytic choriomeningitis virus (LCMV) transgenic model of virus-induced insulin-dependent diabetes mellitus (IDDM) was chosen, and a double transgenic mouse model was created. Both a LCMV transgene [nucleoprotein (NP) or glycoprotein (GP)] and the Ad E3 transgenes were expressed in β cells (RIP-LCMV × RIP-E3 mice). Previously it had been shown that RIP-LCMV transgenic mice do not manifest disease until infected with LCMV, at which time unresponsiveness to the viral GP or NP (self)-antigens is broken, and the mice develop IDDM (7–11). The disease is accompanied by antiviral CTL, which infiltrate the islets and trigger β cell destruction, and is characterized by hyperglycemia, hypoinsulinemia, and infiltration of $CD4^+$, $CD8^+$, and B lymphocytes, as well as dendritic cells into the islets of Langerhans (7, 8, 11). Up-regulation of class I MHC molecules on β cells precedes the overt manifestation of IDDM, which does not occur in the absence of class I MHC molecules, $CD8^+$ CTL, or γ-interferon (12). TNF-α also has been shown to enhance disease in RIP-LCMV-induced IDDM (13).

Two distinct RIP-LCMV models for IDDM have been generated. In the first, the viral transgene (LCMV-GP) is expressed only in the β cells, a rapid-onset IDDM occurs at 10 to 14 days postinfection, and antiviral (self) $CD8^+$ T cells, but not $CD4^+$ T cells, are required (7). In the second model, the transgene (LCMV-NP) is expressed in both the β cells and the thymus, resulting in a slow-onset IDDM. Because high-affinity antiviral CTL are deleted, IDDM takes 1 to 6 months to develop, and the time depends on the host MHC haplotype. CTL found in the periphery are of lower affinity (7, 14), and both $CD4^+$ and $CD8^+$ lymphocytes are required for development of IDDM. In both models, the initiating LCMV-specific anti-self CTL response is restricted by the D^b class I MHC allele (15).

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Abbreviations: Ad, adenovirus; CTL, cytotoxic T lymphocytes; E3, adenovirus early transcription region 3; GP, glycoprotein; IDDM, insulin-dependent diabetes mellitus; LCMV, lymphocytic choriomeningitis virus; MHC, major histocompatibility complex; NP, nucleoprotein; RIP, rat insulin promoter; TNF-α, tumor necrosis factor α.

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Our findings demonstrate that the Ad E3 genes expressed locally as transgenes in β cells can abort the development of both LCMV-NP and LCMV-GP-induced autoimmune diabetes. This occurs in spite of the presence in the spleen of CTLs that can destroy islets containing LCMV polypeptides in the absence of Ad E3 genes. Thus, both rapid- and slow-onset IDDM after LCMV infection are prevented in these mice, without affecting the systemic immune functions or autoimmune responses of the host. These findings have important implications for understanding autoimmunity and perhaps for therapy of IDDM. For example, the Ad E3 genes that we previously have shown to facilitate survival of allogeneic transplanted islets (5) also might be able to confer resistance to the continuous autoimmune destruction that might compromise the viability of transplanted islets in type I diabetes.

MATERIALS AND METHODS

Transgenic Lines and Viral Strains. *Transgenic lines.* Generation and characterization of RIP-LCMV transgenic mice with rapid-onset (8–14 days) or slow-onset (2–6 months) IDDM after LCMV infection has been described (7). RIP-GP 34–20 (H-2^b) transgenic mice that express the viral GP only in the β cells of the islets were used as a model for rapid-onset IDDM. For slow-onset IDDM, RIP-NP 25–3 (H-2^b) transgenic mice that express the viral NP in the pancreas and the thymus, but not in any other tissue, were used (14). Generation and characterization of transgenic mice expressing the Ad E3 region under control of the RIP has been described by Efrat *et al.* (5). These transgenic mice express detectable levels (by reverse transcription–PCR) of mRNA encoding the Ad type 2 E3 region gp19-kDa, 10.4-kDa, 14.5-kDa, and 14.7-kDa protein. Double transgenic RIP-E3 × RIP-LCMV mice expressing both E3 and LCMV proteins in their β cells were generated by mating H-2^b RIP-E3 with H-2^b RIP-LCMV transgenic mice. Offspring were tested by PCR analysis using LCMV and E3 primers, as described (5, 16, 17).

Virus. Virus stock consisted of LCMV Armstrong strain (Clone 53b) that was plaque-purified three times on Vero cells, and stocks were prepared by a single passage on BHK-21 cells.

Analysis of Blood Glucose. Blood samples were obtained from the retro-orbital plexus of mice, and plasma glucose concentration was determined using ACCUCHECK II (Boehringer Mannheim). Insulin concentrations in the pancreas were assessed by radioimmune assays (11).

Histological and Immunohistochemical Analyses of Tissues. Tissues taken for histological analysis were fixed in 10% zinc formalin and stained with hematoxylin and eosin. Immunohistochemical studies were carried out on 6- to 10-μm freshly frozen cryomicrotome sections (14, 16) of the pancreas to detect expression of class I MHC, D^b, CD4, and CD8. Primary antibodies were applied for 1 hr. These consisted of rat anti-mouse CD4 (clone RM 4–5), anti-CD8 (clone 53–6.7), anti-class I (clone M 1/42), anti-D^b (KH95) (PharMingen and Boehringer Mannheim), and anti-LCMV NP antibody 1.1.3 (mouse IgG2a). After washing in PBS, the secondary antibody [biotinylated goat anti-rat (or anti-mouse) IgG/Vector Laboratories] was applied for 1 hr. Color reaction was developed with sequential treatment using avidin-horseradish peroxidase conjugate (Boehringer Mannheim) and diaminobenzidine-hydrogen peroxide (16, 17).

Immunological assays. *CTL assays.* CTL activity was measured in a 5- to 6-hr *in vitro* ^{51}Cr release assay (18, 19). To judge CTL recognition and lysis, syngeneic or allogeneic target cells were infected with LCMV Armstrong strain (multiplicity of infection 1). Assays used splenic lymphocytes at effector-to-target ratios of 50:1, 25:1, and 12.5:1, or secondary CTL lines at ratios of 5:1, 2.5:1, and 1:1. To determine CTL activity after secondary stimulation, spleen cells or lymphocytes from the pancreas harvested from mice 60 days after primary inoculation with 1×10^5 pfu/0.1 ml saline LCMV i.p. were incubated with MHC-matched, irradiated, LCMV-infected macrophages in the presence of T cell growth factor and irradiated syngeneic spleen feeder cells for 5 days (16, 17).

Recovery of pancreatic lymphocytes. Lymphocytes were isolated from the pancreas as described (16, 17). Briefly, tissues obtained from transgenic mice were freed from fat and surrounding lymphoid tissue. After collagenase digestion, lymphoid cells were purified through a Ficoll/Hypaque gradient.

Phenotyping and sorting of lymphocytes. Cultured lymphocytes were phenotyped by FACS analysis using mAbs to murine CD4 (rat mAb YTS 191.1.1) and CD8 (rat mAb YTS 169.4.2) (16, 17, 20).

RESULTS

Expression of Ad E3 genes in β Cells Prevents Virus-Induced IDDM in RIP-LCMV Transgenic Mice. The experiments depicted in Fig. 1 indicate that 80% of RIP LCMV-GP (Fig. 1*A*) and 100% of RIP LCMV-NP (Fig. 1*B*) transgenic mice infected with LCMV developed diabetes, whereas none of the uninfected mice developed the disease (data not shown). When the Ad E3 genes were coexpressed with the LCMV-NP or LCMV-GP transgene in β cells, and the animals were infected with LCMV, none of the double transgenic mice (or those with the Ad E3 transgenes alone) developed diabetes over an 8-month observation period. There was no effect of the Ad E3 genes on the expression of the LCMV transgene as demonstrated by NP immunohistochemistry analysis (Fig. 2).

Self-Reactive LCMV-Specific CTL Are Not Found in Islets of RIP-E3 × RIP-LCMV Double Transgenic Mice, But Are

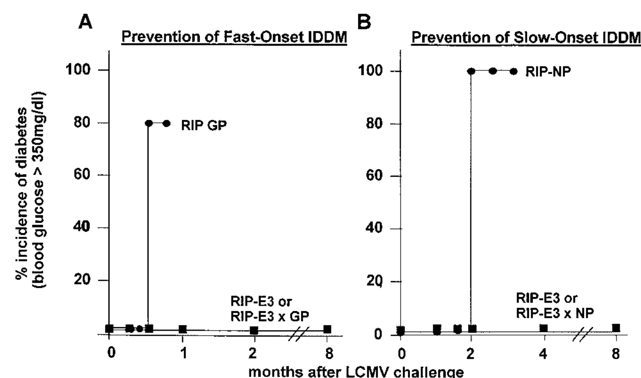

FIG. 1. Expression of Ad E3 genes in β cells prevents LCMV-induced IDDM. Each of four groups (RIP-LCMV, RIP-E3, RIP-LCMV × RIP-E3, and nontransgenic littermates) consisted of 10 mice, five of which were infected with LCMV between 6 and 8 weeks of age. Blood glucose was measured at indicated intervals after infection with 1×10^5 pfu/0.1 ml saline LCMV i.p. as described in *Materials and Methods*. (*A*) The incidence of fast-onset IDDM in RIP-GP (●) transgenic mice was compared with the incidence found in RIP-E3 × GP (■) double transgenic mice or single transgenic RIP-E3 controls. (*B*) The incidence of slow-onset IDDM in RIP-NP (●) mice was compared with the incidence found in RIP-E3 × NP (■) double transgenic mice or single transgenic RIP-E3 controls. (*A*) Both males and females were analyzed. Nontransgenic controls did not show any evidence of diabetes in either the LCMV-infected or uninfected group (data not shown). Diabetes was defined as blood glucose values exceeding 350 mg/dl and pancreatic insulin levels lower than 10 ng/mg tissue. None of the nondiabetic mice displayed blood glucose values above 200 mg/dl. Higher viral dosages than 1×10^5 pfu/0.1 ml saline given to double transgenic RIP-E3 × GP mice did not result in IDDM. (*B*) Only female mice were analyzed, because late-onset (after 6 months of age) spontaneous IDDM was observed in RIP-E3 × NP double transgenic male mice without LCMV infection. None of the other uninfected animals in these groups studied developed IDDM during the 10-month observation period.

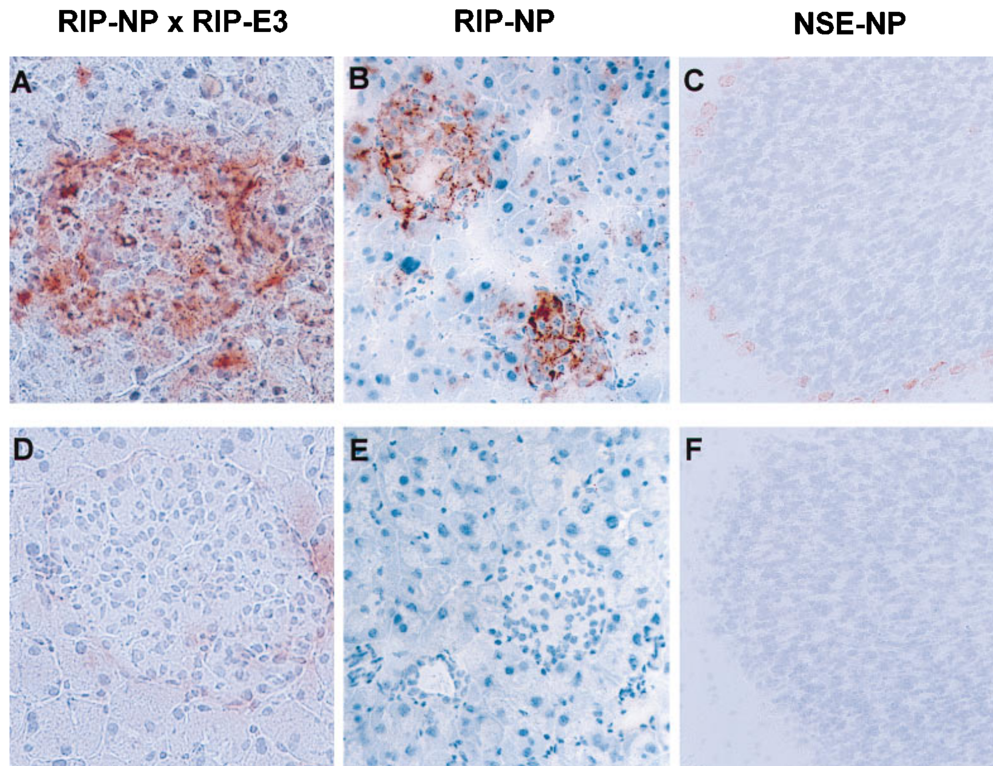

FIG. 2. Expression of E3 transgenes does not affect the expression of LCMV transgenic proteins in β cells. (*A*) An islet from a RIP-E3 × RIP-LCMV-NP double transgenic mouse stained for LCMV-NP protein with antibody 1.1.3., the control of an adjacent section stained without primary antibody is shown in *D*. Equal expression of the LCMV-NP transgene was noted in a RIP-LCMV-NP single transgenic mouse without expression of the E3 proteins (*B*). No NP expression was recorded in an islet of a RIP-LCMV-GP transgenic mouse (*E*). The selectivity of the LCMV-NP immunostaining was demonstrated by using brain sections from a transgenic mouse expressing LCMV-NP under control of the neuron specific enolase promoter (NSE) in cerebellar pyramidal neurons (*C*; without primary antibody, *F*). Immunostaining for LCMV-NP was performed as described in *Materials and Methods*. (*A, C, D,* and *F*) × 400; (*B* and *E*) × 200.

Present in Normal Numbers in Spleens After LCMV Infection. All groups of transgenic mice generated primary and secondary/memory (Table 1) LCMV-specific CTL after LCMV infection. Similar levels of CTL were recovered from spleens at 60 days after LCMV infection in both RIP-E3 × RIP-LCMV mice that did not develop diabetes and in RIP-LCMV transgenic mice that developed IDDM (10). These same patterns were evident as early as 7 days postinfection except that a delay occurred in developing CTL to LCMV-NP. As anticipated (7), RIP-NP H-2^b single transgenic or double transgenic mice generate LCMV-NP-specific CTL with lower activities at 7 days than those found in the corresponding LCMV-GP animals (Table 1). This occurs because mice of the RIP-NP line express the LCMV transgene in their thymuses and delete high-affinity LCMV-NP-specific CTL through negative selection. As a consequence, IDDM develops much slower (Fig. 1).

However, lymphocytes recovered in association with the islets of double transgenic RIP-E3 × RIP-LCMV mice that did not develop IDDM displayed no specific anti-LCMV CTL activity, whereas those recovered from the pancreas of single RIP-LCMV transgenic mice did demonstrate anti-LCMV CTL activity.

E3 Genes Reduce Infiltration of Islets by Lymphocytes in LCMV-Infected RIP-E3 × RIP-LCMV Double Transgenic Mice. Pancreas from nondiabetic double transgenic RIP-E3 × RIP-LCMV and diabetic RIP-LCMV single transgenic mice was obtained 60 days post-LCMV infection and analyzed histologically (Fig. 3 *A* and *B*) and immunohistochemically (Fig. 3 *C-F*). Infiltration of T lymphocytes or dendritic cells into the islets was not observed in double transgenic RIP-E3 × RIP-LCMV mice (5 of 5 mice studied, Fig. 3 *A*, *C*, and *E*). In contrast, such infiltration was seen in all single transgenic RIP-LCMV mice (10 of 10 studied, Fig. 3 *B*, *D*, and *F*). Interestingly, although lymphocytes did not infiltrate into the islets in RIP-E3 × RIP-LCMV double transgenic mice, they consistently were found in smaller numbers around the islets (peri-insulitis). The overall numbers of $CD4^+$ (Fig. 3 *C* and *D*), $CD8^+$ (Fig. 3 *E* and *F*), and B lymphocytes (not shown) found around the islets were lower in double transgenic mice without IDDM, as compared with the numbers of lymphocytes found infiltrating into the islets in diabetic single transgenic RIP-LCMV mice. However, the ratios of $CD4^+$, $CD8^+$, and B lymphocytes found around the islets in double transgenic mice were similar to those found in the islets of single transgenic RIP-LCMV mice. Thus, E3 expression in the β cells prevented infiltration and accumulation of lymphocytes within the islets, but not accumulation of small numbers of inflammatory cells around the islets; however, the peri-insulitis was not associated with injury to β cells.

Expression of E3 Genes in β Cells Prevents Up-Regulation of Class D^b MHC. Our previous studies (12, 16) and those of others (10, 21, 22) showed that class I MHC expression on islet cells is required for IDDM and usually is found in inflammatory islet lesions. Thus, the absence of T lymphocytes in the target area (β cells in the islets) was compatible with a defect in antigen presentation. Indeed, the failure of up-regulation of class I MHC expression was caused by expression of the Ad E3 gene products. This was demonstrated by immunohistochemical analysis of MHC molecules (Fig. 4). Up-regulation of the class I MHC allele D^b occurred in LCMV-infected single transgenic RIP-LCMV mice (Fig. 4*B*), but not in double transgenic RIP-E3 × RIP-LCMV mice (Fig. 4*A*). Up-regulation of other MHC alleles (presumably K^b) could be detected in the islets of double transgenic mice by staining for a common class I MHC epitope; however, the levels in these

Table 1. Expression of E3 genes in β-cells of RIP-LCMV H-2^b transgenic mice reduces the anti-self (viral) CTL response locally in the islets of Langerhans but does not affect the systemic CTL response measured in the spleen

Transgenic mice (H-2^b)	Days post LCMV infection	Origin	E:T	^{51}Cr release (%) from target cells infected with H-2^b LCMV	H-2^b vvGP	H-2^b vvNP	H-2^d LCMV	IDDM
RIP-E3	7	Spleen	50:1	64 ± 5	33 ± 6	23 ± 6	0	No
RIP-GP	7	Spleen	50:1	55 ± 4	28 ± 7	26 ± 8	3	No
RIP-E3 × RIP-GP	7	Spleen	50:1	48 ± 9	30 ± 6	30 ± 7	2	No
RIP-NP	7	Spleen	50:1	38 ± 6	20 ± 3	1 ± 1	0	No
RIP-E3 × RIP-NP	7	Spleen	50:1	75 ± 9	38 ± 6	4 ± 3	1	No
RIP-E3	60	Spleen	5:1	46 ± 9	22 ± 8	29 ± 4	0	No
		Pancreas			no CTL in pancreas			
RIP-GP	60	Spleen	5:1	40 ± 11	25 ± 6	ND	0	Yes
		Pancreas	5:1	40 ± 3	20 ± 8	40 ± 7	2	Yes
RIP-E3 × RIP-GP	60	Spleen	5:1	55 ± 7	23 ± 4	49 ± 4	0	No
		Pancreas	5:1	2 ± 1	3 ± 1	0	0	No
RIP-NP	60	Spleen	5:1	38 ± 8	33 ± 7	34 ± 8	3	Yes
		Pancreas	5:1	28 ± 8	20 ± 6	24 ± 8	0	Yes
RIP-E3 × RIP-NP	60	Spleen	5:1	35 ± 5	40 ± 6	36 ± 9	0	No
		Pancreas	5:1	0	4 ± 2	3 ± 1	0	No

CTL assays were performed as described in *Materials and Methods*. Target cells were MC57 (H-2^b) or BALB/c17 (H-2^d) fibroblasts uninfected or infected with the Armstrong strain of LCMV or vaccinia viruses expressing the complete LCMV-NP (vvNP) or LCMV-GP (vvGP) proteins. Background lysis of uninfected cells was lower than 5% in all assays and was subtracted from the lysis (^{51}Cr release) values shown. Five mice were tested per group and the mean ±1 SE is displayed. All groups of mice generated normal (14, 19) levels of anti-LCMV primary CTL, which were measured directly *ex vivo* on day 7 post-LCMV infection with 1×10^5 pfu i.p. Secondary CTL activity in spleens was determined on day 60 after infection, after a 5-6 day *in vitro* stimulation in the presence of LCMV antigen and antigen-presenting cells as described in *Materials and Methods*. CTLs isolated from the islets of Langerhans were stimulated *in vitro* for 15 days in the presence of syngeneic-irradiated antigen-presenting cells expressing LCMV antigens and feeder cells (see *Materials and Methods*). Effector/target (E/T) ratios are on display.

mice were lower compared with single transgenic RIP-LCMV islets (Fig. 4 *C* and *D*).

DISCUSSION

Using the RIP-LCMV model of virus-induced diabetes, we show that expression of Ad E3 genes in the pancreas can prevent autoimmune diabetes caused by a response against a (viral) self-antigen. As a probable mechanism we find that up-regulation of the class I MHC D^b allele, which usually is observed in the islets of RIP-LCMV mice after initiation of IDDM by LCMV infection, was selectively absent in mice expressing the Ad E3 genes.

Expression of the Ad E3 gene complex in β cells prevents virus-induced IDDM in the presence of an antigen-specific trigger. This occurs without influencing the systemic (splenic) anti-viral (self) immune response (Table 1), which is responsible for initiating β cell destruction in RIP-LCMV single transgenic mice (11, 12, 14, 17). This indicates that regulation takes place locally in the islets of Langerhans. All mice expressing the E3 genes show absence of lymphocytes infiltration into their islets (Fig. 3); however, lymphocytes could be detected around the islets in these mice. These lymphocytes recovered from the peri-islet region fail to show anti-self (viral) CTL activity (Table 1). In contrast, anti-self CTLs are easily demonstrated among T lymphocytes recovered from islet lesions of RIP-LCMV transgenic mice that do not express E3 in their β cells. Whether the lack of anti-self CTL activity in the islets of the double transgenic mice occurs because the CTL had undergone apoptosis currently is being studied. Alternatively, anti-self CTLs may not be retained in the islets because they lack local activation that usually is provided by up-regulation of class I MHC D^b-mediated antigen presentation.

Thus, our findings show that one reason for the prevention of IDDM in double transgenic mice expressing the E3 genes is a selective inhibition of the up-regulation of D^b on the β cell surface during induction of IDDM by LCMV infection (Fig. 4). Up-regulation of D^b and other H-2^b MHC alleles usually is seen as early as 7–14 days after LCMV infection in RIP-LCMV single transgenic mice before the onset of IDDM (ref. 12; Fig. 4). Our previous studies (12) have shown that class I MHC expression on islet cells is required for IDDM and usually found in inflammatory islet lesions. This is likely the case, because MHC expression is needed for the presentation of self-antigens on β cells. Thus, a selective reduction of D^b

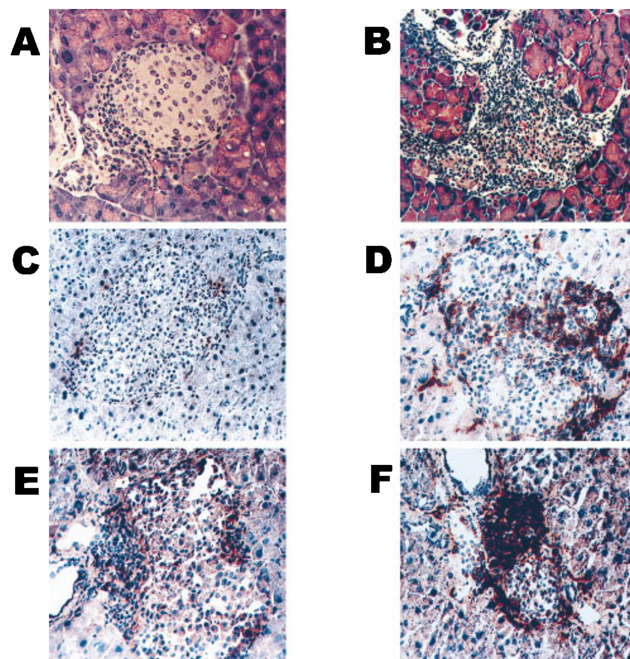

FIG. 3. Histological analysis of insulitis in islets of RIP-NP transgenic mice or RIP-NP × RIP E3 double transgenic mice 60 days post-LCMV infection. (*A*, *C*, and *E*) Pancreatic sections from RIP-NP × RIP-E3 double transgenic mice that received 1×10^5 pfu/0.1 ml saline LCMV and did not develop IDDM. (*B*, *D*, and *F*) Sections from virus-infected RIP-NP single transgenic mice that developed IDDM. (*A* and *B*) Hematoxylin-eosin (HE) staining. (*C* and *D*) Immunohistochemical staining for $CD4^+$. (*E* and *F*) Immunohistochemical staining for $CD8^+$. (See *Materials and Methods*.) (*A*) × 400. (*B, C, D, E,* and *F*) × 200.

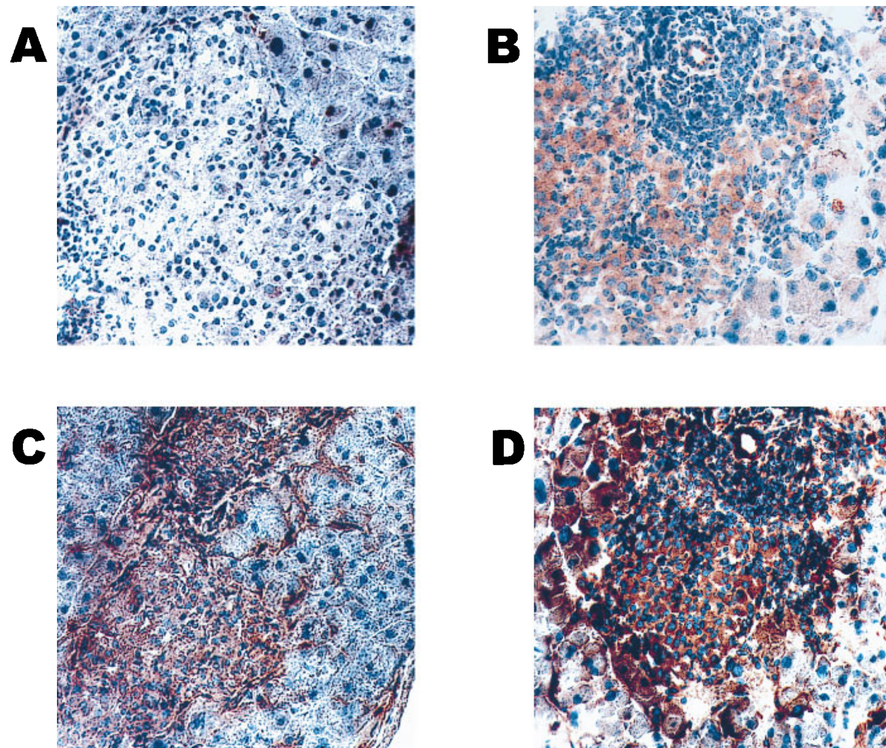

FIG. 4. Immunohistochemical analysis of class I MHC (D^b) and total MHC class I in islets from RIP-NP transgenic mice or RIP-NP × RIP E3 double transgenic mice 60 days post-LCMV infection. Expression of the class I MHC D^b allele (*A* and *B*) and overall MHC class I (*C* and *D*) was compared between single transgenic RIP-NP mice (*B* and *D*) that developed IDDM and double transgenic RIP-NP × RIP-E3 mice without IDDM (*A* and *C*). × 200.

expression probably leads to insufficient expression of the self (viral) antigen (or a LCMV-derived peptide) on the surface of β cells. Consequently, killing of islet cells by LCMV-specific CTL, a prerequisite for the induction of autoimmune diabetes in RIP-LCMV transgenic mice is prevented (11, 12, 14).

Our data are in agreement with *in vitro* studies documenting that gp19 binds well to D^b and reduces its cell surface expression, but has less effect on K^b (2, 3, 5). The up-regulation of MHC molecules other than D^b occurs in islets of double transgenic mice without leading to IDDM. This is because the other class I MHC alleles are unable to present LCMV peptides that are recognized by CTL or do it inefficiently. The major H-2^b-restricted CTL response to LCMV was shown to be directed primarily to three immunodominant epitopes presented by D^b, i.e., GP1 (amino acids 34–42), GP2 (amino acids 276–286), and NP (amino acids 386–405) (18, 19). Only the GP1 epitope has a K^b motif and can be presented by K^b, but the D^b-restricted response dominates the K^b response (M.G.v.H., J. E. Gairin, and M.B.A.O., unpublished observations). Hence, the few CTL present around the islets of double transgenic RIP-LCMV × RIP-E3 mice likely are restricted by K^b, but by themselves are unable to destroy sufficient numbers of islet cells to cause IDDM.

In vitro studies in other experimental viral models also have demonstrated class I MHC immune-regulatory effects, which interfere with antigen presentation (23, 24). For example, human herpes simplex virus ICP47 and human cytomegalovirus U2 and U12 proteins decrease class I MHC at the cell surface by mechanisms that are different from those of the Ad gp19. To our knowledge, none of these genes have been used in an attempt to prevent IDDM.

Other approaches to down-regulate class I MHC presentation in islet cells have been reported (25, 26). In β2-microglobulin gene knockout experiments, all class I MHC recognition is aborted, not only because of deletion of the target, but also because such animals lack effector T cells. Although such an approach has been shown to be useful in facilitating murine islet transplantation, the Ad E3-based approach has several distinct advantages. First, these Ad genes can be selectively expressed in β cells, allowing the creation of a state of immunologic ignorance only of a specific target within the context of a fully competent immune system. In addition, the effects of the Ad gp19 gene are dominant in its control of class I MHC, and this allows it to be added to any cell, including human islets. In contrast, the gene deletion approach thus far has been successful using only murine embryonic stem cells.

In addition to gp19, the Ad E3 region encodes a 14.7-kDa protein and a complex of 10.4-kDa and 14.5-kDa proteins that modulate TNF-α-induced cytolysis (2, 6). Control of the TNF-α effect might constitute another mechanism by which E3 genes can regulate an inflammatory reaction, because TNF-α has been shown to enhance IDDM in the RIP-LCMV model (13) and other autoimmune diseases (27). Thus, it is possible that intracellular inhibition of TNF-α-mediated cytotoxicity by the Ad E3 proteins also contributes to the prevention of β cell destruction. The role of the Ad E3 anti-TNF genes in β cell survival in the LCMV autoimmunity model is being approached genetically by remaking transgenic animals containing the Ad E3 genes that have been deleted of the anti-TNF functions.

In conclusion, the Ad E3 genes expressed *in vivo* in islets have been used successfully to prevent autoimmune diabetes. These results extend our previous findings that Ad E3 genes could facilitate allogeneic transplantation (5) and show that target-cell specific effects are clearly segregated from effects on the systemic autoimmune response. Recently, we also have used the Ad E3 genes to down-regulate the immune response (including antibody formation) to Ad gene-therapy vectors and have prolonged survival of foreign gene expression from this source (28). Because the humoral and cell-mediated immune responses have been some of the limiting factors in the

successful use of Ad-based gene delivery vectors (29, 30), overexpression of the E3 genes appears to have controlled these responses and should extend the usefulness of Ad-based gene therapy protocols. It appears from the present study that immunologic control should be effective even if limited only to the target cell without affecting systemic immunity. Thus, the Ad E3 genes have demonstrated potent immunomodulation *in vivo* in three rodent models (transplantation, autoimmunity, and Ad-based gene therapy vectors) that would be useful to pursue in the control of human disease.

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