Adenovirus Early Region 3 Transgenes Expressed in β Cells Prevent Autoimmune Diabetes in Nonobese Diabetic Mice: Effects of Deleting the Adenovirus Death Protein 11.6K

Melissa A. Pierce,1 Anton Svetlanov,2 Marshall S. Horwitz,2 and David V. Serreze1*

The Jackson Laboratory, Bar Harbor, Maine,1 and Department of Microbiology and Immunology, Albert Einstein College of Medicine, Bronx, New York2

Received 29 April 2004/Accepted 5 August 2004

The incidence of type 1 diabetes (T1D) is decreased in nonobese diabetic mice expressing the complete cassette of adenovirus early region 3 (E3) immunomodulating genes in pancreatic β cells. Embedded among the antiapoptotic E3 genes is one encoding an adenovirus death protein (ADP), which contributes to release of virion particles by promoting cell lysis. Because removal of this proapoptotic protein might have further enhanced the ability of E3 proteins to prevent T1D, an ADP-inactivated E3 construct was tested. Significantly, deletion of ADP did not improve the diabetes-protective effect of an E3 gene cassette.

Nonobese diabetic (NOD) mice provide a standard, well-studied model of type 1 diabetes (T1D), developing disease similarly to humans as a result of a polygenically activated autoreactive T-cell response that destroys insulin-producing pancreatic β cells (reviewed in reference 12). In both genera, T1D requires contributions from both major histocompatibility complex (MHC) class I and class II-restricted T cells (reviewed in reference 12). Pancreatic β cells express only MHC class I molecules (9) and thus can directly present internally derived peptides to autoreactive cytotoxic CD8 T cells. MHC class II expression is largely restricted to hematopoietically derived antigen-presenting cells. Diabetogenic CD4 T cells recognize MHC class II bound peptides that antigen-presenting cells process from internalized β-cell proteins. Activated CD4 T cells produce cytokines, providing a source of help for triggering cytotoxic CD8 T cells. Cytokines such as interleukin 1β, tumor necrosis factor alpha (TNF-α), and gamma interferon also directly contribute to β-cell destruction (13). Furthermore, these cytokines collectively up-regulate Fas levels on the surfaces of β cells, increasing their sensitivity to apoptotic death induced by FasL molecules expressed by both diabetogenic CD4 and CD8 T cells (1, 2). Hence, any successful genetic engineering approach for preventing T1D must have the ability to thwart this wide collection of autoimmune mechanisms. One way β cells in NOD mice can be shielded from such a broad array of autoimmune effector mechanisms is to transgenically express the multiple immunoregulatory genes comprising the early region 3 (E3) of the adenovirus genome (AdE3) (10).

Adenoviruses use at least five E3-encoded molecules to evade host immune responses (reviewed in reference 8). The E3-encoded gp19K protein down-regulates MHC class I presentation of antigenic peptides by binding and retaining class I molecules in the endoplasmic reticulum and preventing tapasin processing of peptides that bind to class I molecules (8). These processes limit recognition of infected cells by MHC class I-restricted cytotoxic CD8 T cells. E3 also encodes the 14,700-molecular-weight (14.7K), 10.4K, and 14.5K proteins, which inhibit TNF-α and Fas-mediated apoptosis of infected cells.

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* Corresponding author. Mailing address: The Jackson Laboratory, 600 Main St., Bar Harbor, ME 04609. Phone: (207) 288-6403. Fax: (207) 288-6079. E-mail: dvs@jax.org.
While it is not fully clear how the 14.7K protein inhibits apoptosis, it has been shown to block cytosolic activation of phospholipase A2, a molecule associated with arachidonic acid release. In addition, the 14.7K protein interacts with FADD-like interleukin-1 \( \beta \)-converting enzyme, which is a shared effector caspase for both TNF receptor 1 and Fas (11). The E3-encoded 10.4K and 14.5K proteins form a heterotrimer (one molecule of the 14.5K protein and two molecules of the 10.4K protein) that inhibits apoptosis by internalizing cell surface Fas receptors and subsequently degrading them in lysosomes (8). Activation of the NF-kB transcription factor is also inhibited by the 10.4K/14.5K proteins (6). The 11.6K protein or adenovirus death protein (ADP) differs from other E3-encoded molecules by the fact that (i) it is synthesized at late stages of viral infection due to its regulation by a strong late promoter, and (ii) it mediates cellular lysis to promote virion release (14, 17).

We have found that among the E3 genes, those inhibiting cytokine-induced apoptosis (encoding the 14.7K, 10.4K, and 14.5K proteins) have the most potent TID-inhibitory effects when jointly expressed as transgenes in NOD \( \beta \) cells (10). However, the question remained whether the potentially harmful proapoptotic effects of the 11.6K gene were limiting the full potential of an E3 cassette to elicit diabetes protection when expressed in NOD \( \beta \) cells. To address this hypothesis, a rat insulin promoter (RIP)-regulated E3 construct was engineered with point mutations in the first two methionines of the 11.6K open reading frame, changing these residues to leucines, and inhibiting ADP synthesis (designated RIP-DL734). These mutations had no effect on the synthesis of other E3 proteins when studied previously (15). AdE3 DNA between the BspE1 and NdeI sites was isolated from Ad-DL734 (14), processed as previously described for the Ad type 2 E3 (Ad2E3) constructs (3), and transgenically introduced directly into fertilized NOD oocytes (Cell Biology and Microinjection Service; The Jackson Laboratory, Bar Harbor, Maine). RIP-DL734/NOD litters were screened for transgenic founders by using the PCR protocol used to detect the original E3 construct (4).

Transgenic AdE3 protein expression is difficult to detect (4). Therefore, we confirmed E3 gene expression by the presence of appropriate mRNA transcripts in pancreatic islets isolated as previously described (7, 10) from RIPE3/NOD, RIP-DL734/NOD, and standard NOD mice. Islets from four mice per strain were pooled, and RNA was extracted and treated with DNase 1 by using the RNAqueous-4PCR kit (Ambion Inc., Austin, Tex.). RNA was reverse transcribed by using the RETROscript kit (Ambion Inc.). The DL734/NOD mice expressed the same pattern of E3 region genes (encoding the gp19K, 10.4K, 14.5K, and 14.7K proteins) in islets as seen in the original RIPE3/NOD stock (Fig. 1). Although the RIPE3/NOD stock was generated with a wild-type Ad2E3 construct and DL734/NOD mice contain an Ad2/5 hybrid E3 region, the E3 gene products of these highly homologous serotypes appear to have identical functions and have been used interchangeably. As a control, \( \beta \)-actin (348 bp) mRNA transcripts were confirmed in the islets of all three strains (Fig. 1), using the previously described primers (10). Transcripts were resolved in 1.5% agarose gels and visualized by ethidium bromide staining.

In order to confirm the presence of the mutated 11.6K mRNA in RIP-DL734/NOD islets, the reverse transcriptase...
product was purified by using the QIAquick gel extraction kit (QIAGEN, Inc., Valencia, Calif.) and sequenced (DNA Sequencing Service; The Jackson Laboratory). Sequence analyses revealed 2 A→T transversions that converted the first two methionines in the open reading frame of the 11.6K mRNA transcript to leucine residues in islets from DL734/NOD mice but not RIPE3/NOD mice (Fig. 2).

Diabetes development was assessed in female RIPE3/NOD mice, DL734/NOD mice, and nontransgenic littermate controls by weekly monitoring of glycosuria, using Ames Diastix (Miles Diagnostics, Elkart, Ind.). Values of >3 were considered diagnostic of diabetes development. While the diabetes frequency for the first two groups were significantly less than that observed through 30 weeks of age for NOD control females (81.3%), the final disease incidence in the DL734/NOD (33.3%) and RIPE3/NOD (36.0%) stocks did not differ (Fig. 3). Compared to the NOD controls, the kinetics of diabetes development was significantly slower in both RIPE3/NOD mice (P < 0.0001) and DL734/NOD mice (P < 0.05). Collectively, these results demonstrate that blocking 11.6K protein production did not enhance the ability of an E3 gene cassette expressed in NOD pancreatic β cells to elicit diabetes protection.

The ADP gene has been studied independently of other AdE3 genes in models to enhance tumor-specific killing (16). In such experiments, the intent was to include ADP to enhance tumor cell killing, while in our model, we proposed that removal might have enhanced β-cell survival. Because β-cell destruction in T1D is dependent on MHC class I antigen presentation as well as cytokine-induced apoptosis, E3 genes provide an attractive source for genetic engineering of β-cell transplants. However, our data indicate that the presence of the proapoptotic 11.6K gene does not compromise the ability of other E3 genes encoding antiapoptotic molecules to shield β cells from T1D-inducing autoimmune responses.

This work was supported by NIH P01-DK52956 (M.S.H.) and NIH grants DK46266 and DK51090 as well as grants from the Juvenile Diabetes Research Foundation (D.V.S.). We thank William Wold and Ann Tolleson of St. Louis University for providing the Ad-DL734 virus from which the mutant E3 cassette was cloned.

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