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*J Immunol* 2001; 166:4773-4779; doi: 10.4049/jimmunol.166.7.4773

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Specific Immunotherapy by Genetically Engineered APCs: The “Guided Missile” Strategy

Bo Wu,* Jian-Ming Wu,* Alexei Miagkov,* Robert N. Adams,* Hyam I. Levitsky,† and Daniel B. Drachman2*†

We tested the hypothesis that APCs genetically engineered to present an Ag and to express Fas ligand (FasL) simultaneously can target and eliminate Ag-specific T cells. Transgenic T cells specific for influenza hemagglutinin (HA) were used as targets. We prepared recombinant vaccinia virus vectors (VVV) to transfer the gene constructs individually or simultaneously into APCs. We prevented unwanted viral replication by attenuating the VVVs with psoralen-UV light treatment. For presentation of the HA Ag, APCs were transduced with cDNA for HA flanked by sequences of the lysosome-associated membrane protein that direct efficient processing and presentation of the Ag by APCs. As a “warhead” for the APCs, we transduced them with the gene for FasL, which induces apoptosis of Fas-expressing activated T cells. To protect the transduced APCs from self-destruction by FasL, we transferred cDNA for a truncated form of Fas-associated death domain, which inhibits Fas-mediated cell death. Our results show that the engineered APCs effectively expressed the genes of interest. APCs transduced with VVV carrying all three gene constructs specifically killed HA-transgenic T cells in culture. Coculture with T cells specific for an unrelated Ag (OVA) had no significant effect. Our in vitro findings show that APCs can be genetically engineered to target and kill Ag-specific T cells and represent a promising novel strategy for the specific treatment of autoimmune diseases. The Journal of Immunology, 2001, 166: 4773–4779.

One of the major goals of immunotherapy is to eliminate specific immune responses, without otherwise affecting the immune system (1). We have developed a powerful new strategy using genetically engineered APCs as “guided missiles” to target and eliminate Ag-specific T cells. Because virtually all naturally occurring immune responses are not only highly heterogeneous but also unique to the individual (2–5), it is necessary to devise a method capable of targeting the entire spectrum of each individual’s unique Ag-specific T cell repertoire. The present strategy is based on the natural ability of APCs from a given individual to process and present the Ag so as to target that individual’s entire repertoire of Ag-specific T cells, no matter how heterogeneous it may be. For T cell targeting in this study, we have engineered APCs with a gene construct coding for the model Ag influenza hemagglutinin (HA)1 fused to signals that direct the APCs to process and present the Ag in association with MHC class II (i.e., the lysosome-associated membrane protein, LAMP-1) (6–8). As a “warhead,” we have engineered the APCs to express Fas ligand (FasL). When FasL interacts with Fas, which is highly expressed on the surface membranes of activated T cells, it induces apoptosis and death of the T cells (9, 10). We have protected the APCs from self-destruction by the FasL by inserting a third gene, expressing a truncated form of the Fas-associated death domain (TrFADD), which acts in a dominant negative fashion to prevent Fas-FasL-induced apoptosis of the APCs (11, 12). To transfer the three gene constructs individually or simultaneously into APCs, we have prepared a series of recombinant vaccinia virus vectors (VVV) (13–15). Attenuation of the VVV (by treatment with psoralen and UV light (PUVA)) prevents replication of the virus but permits efficient infection of APCs and expression of the VVV-transferred gene products (16).

In the present study, we have explored the ability of APCs with VVV-transferred genes to target and eliminate Ag-specific T cells, using a murine transgenic T cell model specific for influenza HA (17) in vitro. Our findings indicate that the APCs express all three gene products and effectively and specifically induce apoptosis of HA-specific T cells, while sparing T cells of other specificities.

Materials and Methods

Mice

BALB/c transgenic mice expressing an αβ TCR specific for the HA epitope (18) 110–120 were bred and maintained in the animal care facilities at the Johns Hopkins University. BALB/c transgenic mice expressing an αβ TCR cell receptor specific for OVA (DO11.10 strain (19)) were a gift of Dr. K. Murphy (Washington University, St. Louis, MO). Wild-type (wt) BALB/c mice (8–12 wk old) were purchased from The Jackson Laboratory (Bar Harbor, ME) or the National Cancer Institute (Frederick, MD). All animal experiments were performed in accordance with protocols approved by the Animal Care and Use Committee of the Johns Hopkins University School of Medicine.

Cell lines

A20, human TK−, and CV-1 cell lines were purchased from American Type Culture Collection (Manassas, VA). The MC57G mouse fibroblast cell line was provided by Dr. D. Pardoll (Johns Hopkins University).

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Received for publication November 8, 2000. Accepted for publication January 26, 2001.

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1 This work was supported in part by National Institutes of Health Grants NS07368 and 1R01NS37205 and by the Muscular Dystrophy Association. Generous support was provided by the C. W. Parke Family Foundation, the Ann and Donald Brown Family Foundation, and the Eleanor Dennead Ingram Foundation.

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3 Abbreviations used in this paper: HA, hemagglutinin (influenza); BrdU, bromodeoxyuridine; FADD, Fas-associated death domain; FasL, Fas ligand; LAMP-1, lysosome-associated membrane protein-1; PUVA, psoralen plus UV light; TK, thymidine kinase; TrFADD, truncated FADD; VV, vaccinia virus; VVV, vaccinia virus vector; wt, wild type.
**Insertion of genes into VVV**

The genes of interest were first ligated into appropriate transfer plasmids and then transfected into the vaccinia virus (VV) by homologous recombination in predetermined loci within the viral genome (14). To insert more than one gene, different transfer plasmids are used sequentially, inserted into different loci, and using different methods of selection at each successive step of recombination (13, 15). In these studies, we have inserted up to three genes in a single VV (Fig. 1) and have demonstrated that all of the genes are efficiently expressed by infection of APCs. The first gene (HA-LAMP), which induces processing and presentation of HA, was inserted in a transfer plasmid that recombines in the J2R region of the virus and disrupts the vaccinia thymidine kinase (TK) gene. Recombined VVV was selected with medium containing bromodeoxyuridine (BrdU), which is lethal to cells that do not express TK. VV with a cDNA construct for HA fused to LAMP-1 was a generous gift of D. Pardoll. The second gene (FasL) was inserted in a transfer plasmid that recombines in the HSV-1 genome and induces expression of β-glucuronidase (15). β-Glucuronidase converts the chromogenic substrate 5-bromo-4-chloro-3-indolyl-β-glucuronide to a blue color that is used for selection of plaques.

**Construction of transfer plasmids**

We designed primers for FasL (based on published sequences (20)), amplified FasL cDNA from human lymphocyte cDNA library by PCR, and verified it by sequencing. The FasL cDNA was cloned into the pcDNA3.1 plasmid. The FasL fragment was amplified by PCR using a pair of primers that were designed to anchor on the pcDNA3.1 plasmid and to have BamHI restriction sites at both ends. The primer sequences were as follows: sense primer, ATATGCATTGGACTGGAGCTACACG and antisense primer, TATAGAATAAGGGAGACCCAAGC. The PCR product was digested with BamHI restriction enzyme and ligated into the pIV113 transfer plasmid (Fig. 1).

**Insertion of genes into VV and selection**

For insertion of genes into VV, CV-1 cells were seeded at 2.5 × 10^6/well in six-well plates in MEM with 10% FBS (13, 15, 21). One day later, the cells were infected with the WR strain of VV at 0.1–1 PFU/cell in 1 ml MEM with 2.5% FBS at 37°C with gentle shaking every 20–30 min for 1–2 h. Transfection of the CV-1 cells with the pSMcM2 plasmid was conducted using the Maximum Efficiency Transfection Method (Life Technologies, Gaithersburg, MD) according to the manufacturer’s recommendation. The transfected cells were harvested, frozen, and thawed three times, and sonicated to release the VVV, and four 10-fold dilutions were made in MEM-2.5. Selection of recombinant VVV was conducted as follows. Cloned TK cells in six-well plates were infected with 1 ml of each dilution of the transfectant by incubation at 37°C for 2 h with gentle rocking after the medium was discarded. Cells were gently overlaid with 3 ml warm 1% LMP agarose in 1× plaque medium (Life Technologies, Gaithersburg, MD) with 5% FBS (HyClone, Logan, UT), 25 μg/ml BrdU (Sigma, St. Louis, MO). The agarose layer was allowed to solidify at room temperature, and the cultures were incubated at 37°C for 2 days. For blue color selection, the cultures were overlaid with 2 ml low melting point (LMP) agarose in 1× plaque medium with 10 mg/ml neutral red and 1/150 volume of 5% bromo-4-chloro-3-indolyl-D-glucuronide (X-Gal) and incubated at 37°C for an additional 1–2 days. Discrete blue plaques were picked, freeze-thawed three times, and used for reselection as above. Three to five rounds of selection were conducted until pure, single plaques were obtained. Individual plaques were picked by PCR and RT-PCR for each recombinant gene before being amplified. Viral stocks were produced in CV-1 cells in T-175 flasks, aliquoted, and stored at −70°C.

For production of recombinant VVV containing a second gene, we used the TK^− transfer plasmid, pTK7.5b (Fig. 1). The procedures for transfection of the CV-1 cells, and TK^− selection were similar to those described above, with the one critical exception that the selection medium contained MTAGG (methotrexate (3 μM), thymidine (15 μM), adenosine (50 μM), guanosine (50 μM), and glycine (10 μM); the methotrexate component selects for TK^− cells), instead of BrdU. As above, three to five rounds of selection were conducted to obtain pure two-gene VVV.

For production of recombinant VVV containing the third gene, we used a β-glucuronidase-expressing “GUS” transfer plasmid (pIV113) containing the late FADD gene, and a selection method that depends on production of color by the expressed marker β-glucuronidase. The procedures for transfection of the CV-1 cells were as described above. The first agarose overlay did not contain any selection reagent. In the second layer, 5-bromo-4-chloro-3-indolyl-β-glucuronide (200 μg/ml; Clontech, Palo Alto, CA) was used for the development of blue color. Because this selection method does not involve negative selection of nonrecombined VVV, five to six rounds of selection were carried out until pure single plaques were obtained.

**In vitro T cell proliferative responses**

Different numbers of lymph node cells or splenocytes were seeded in 96-well plates in complete medium (RPMI with 10% FBS, 5.5 × 10^−3 M 2-ME, 1 mM HEPES buffer, 100 U/ml penicillin, 100 μg/ml streptomycin, and 0.25 μg/ml Fungizone) and incubated at 37°C with 5% CO2 for 2–5 days. Ag or APCs transduced with various VVV, were added to the wells in complete medium (RPMI with 10% FBS, 5.5 × 10^−3 M 2-ME, 1 mM HEPES buffer, 100 U/ml penicillin, 100 μg/ml streptomycin, and 0.25 μg/ml Fungizone). The media were changed every 3 days. The cells were labeled with 1 μCi/well of [3H]TdR for 8–16 h and harvested onto glass fiber filters. Dried glass fiber filters were counted, and the results were expressed as Δcpm ± SEM.

**Flow cytometry**

Ab to human FasL and clonotypic Ab to the DO11.10 OVA-specific T cells (KJ1-26) were purchased from Caltag (Burlingame, CA). All other Abs used for flow cytometry were purchased from Pharmingen (San Diego, CA). Cells (2–10 × 10^6) were dispensed in 96-well plates in 1 × HBSS supplemented with 1% FBS and 0.5% sodium azide. To each well were added 10–20 μl of appropriately diluted Ab solutions, and the mixture was incubated on ice for 30 min. After three washes, 10–20 μl of properly diluted secondary Ab were added and incubated for 30 min. After fixing with Cytofix (Pharmingen), cells were analyzed immediately or within 1 wk using a FACScan/FACSorter (BD Biosciences, San Jose, CA).

**DNA fragmentation induced by FasL expressed by recombinant VV**

The expression of FasL in the MC3T3 mouse fibroblast cells was infected with recombinant VV containing the gene for FasL, or control VV, at 20 multiplicity of infection for 5 h in six-well plates. A20 mouse B lymphoma cells, which express Fas abundantly, were used as target cells after labeling overnight with [3H]Tdr (5 μCi/10^6 cells/ml in 100-mm petri dishes at 37°C).
Labeled A20 cells (10^4 per 1.5-ml tube) were cocultured with different numbers of transduced MC57G cells, as indicated in Fig. 3 at 37°C. Negative controls contained labeled A20 cells alone; positive controls contained labeled A20 cells with added anti-Fas Ab. After 24 h coculture, all tubes were centrifuged at 2500 rpm for 5 min. The supernatants (~200 μl) were collected in scintillation vials with 2.5 ml scintillation fluid. The pellets were further extracted by mixing vigorously with 200 μl 1× TE buffer with 1% Triton and centrifuging at 14,000 rpm. Radioactivity in each supernatant (S), Triton-extracted supernatant (T), both containing fragmented DNA, and pellet (P), containing unfragmented DNA, was counted. The result is expressed as the percentage of total DNA that was fragmented: % of DNA fragmented = ([fragmented DNA (S + T)/total DNA (S + T + P)]) × 100%.

**PUVA attenuation of recombinant VVs**

The following procedure was used to determine the appropriate parameters of PUVA treatment to attenuate each batch of VVV. Viral suspensions were freshly made in the range of 10^8–10^9 PFU/ml in 1× HBSS with 0.1% BSA, and 1 ml was transferred to each 35-mm tissue culture dish. Psoralen (Trioxsalen; Calbiochem, La Jolla, CA) was added to a final concentration of 1–10 μg/ml and incubated at 20°C for 10 min. UV irradiation was conducted in a Stratagene 1800 UV irradiation unit (Stratagene) for 1–10 min. PUVA-treated virus suspensions were used immediately, stored at 4°C for days, or stored in aliquots at −80°C for months. Confluent monolayers of CV-1 cells were infected with 10^5–10^8 PFU virus/well in MEM-2.5 and incubated overnight at 37°C with 5% CO_2_. Plaques were counted after staining with 0.5% crystal violet. CV-1 cells infected with PUVA-treated VVV in another six-well plate were used for RT-PCR. The appropriate PUVA attenuation parameter is defined as the minimal dose of psoralen and shortest UV irradiation time required to eliminate plaque formation (replication), but with preserved production of mRNA as shown by positive RT-PCR results.

**Results**

**Construction of recombinant VVs with FasL and TrFADD**

As described above, we prepared, selected, and amplified recombinant VVV containing the three gene constructs, in five single or multiple combinations, as follows: HA-LAMP-1; FasL; HA-LAMP-1 + FasL; FasL + TrFADD; HA-LAMP-1 + FasL + TrFADD (“three-gene VVV”). Each transfer plasmid was tested by PCR and sequencing for the accuracy of the construct. Each recombinant VVV was tested by PCR for the presence of the appropriate gene construct(s) and by RT-PCR for transcription of mRNA for the recombinated genes. In all cases, the selected VVV contained and expressed the gene constructs of interest (data not shown).

**APCs expressing VVV-transferred HA-LAMP stimulate HA-specific T cells**

Lymph node cells from HA-specific TCR transgenic mice were cocultured with congenic BALB/c mouse splenocytes that had been infected overnight with the HA-LAMP VVV. Cultures were harvested at different time points with the addition of [3 H]TdR overnight for the last 16–18 h (Fig. 2). The results showed pronounced stimulation of the HA-specific T cells. This experiment demonstrated that the product of the VVV-transferred HA-LAMP gene was processed and presented by APCs and stimulated HA-specific T cells to proliferate.

**FasL gene product expressed by recombinant viruses kills Fas+ cells**

Expression of FasL was tested in infection of the MC57G cell line with each FasL-containing VVV overnight at a multiple of infection of 20:1, and staining with PE-labeled specific Ab to FasL. Flow cytometry demonstrated that up to 83% of the MC57G cells expressed FasL (Fig. 3a). To test the function of the FasL gene product expressed by the recombinant viruses, we used Fas-positive A20 B lymphoma cells as targets. A20 cells pulsed with [3 H]TdR were incubated with different numbers of VVV-transduced MC57G cells overnight (see details in Materials and Methods). As shown in Fig. 3b, the FasL gene products expressed by the VVV-infected cells induced apoptosis of the A20 cells. The percentage of DNA that was fragmented varied from 40 to 80%, with different recombinant viruses, contrasted with only 15–25% in A20 cells incubated with control uninfected MC57G cells or MC57G cells infected with wt VV. The results indicate that these different recombinant viruses express strongly functional FasL gene products.

**APCs expressing VVV-transferred “three-gene” products kill HA-specific T cells**

Lymph node cells from HA-specific TCR transgenic mice and from OVA-specific TCR transgenic mice were collected and cultured for 48 h in RPMI 10 with the corresponding Ag, either HA

**FIGURE 2.** Stimulation of TCR-transgenic HA-specific T cells by APCs transduced with the HA-LAMP-1 VVV. T cells from lymph nodes of HA-transgenic mice were enriched by depletion of MHC class II cells by paramagnetic beads. APCs were transduced by infection with attenuated HA-LAMP-1 VVV. Cultures were prepared in 96-well plates with 5 × 10^4 T cells and 5 × 10^3 APCs per well. Unstimulated cultures were used for background counts. At daily intervals from 2 to 7 days, triplicate sets of cultures were pulsed with [3 H]TdR overnight, and incorporated radioactivity was expressed as Δcpm ± SEM. T cells responded vigorously to stimulation with VVV-transduced APCs.

**FIGURE 3.** Expression of FasL by VVV-transduced cells. a, Flow cytometry showing expression of FasL by MC57G fibroblast cells transduced by infection with VVV, stained with PE-labeled Ab to FasL. Dashed line, negative control. b, Functional assay, showing DNA fragmentation of A20 cells incubated overnight with MC57G cells transduced with VVV carrying the FasL gene. Marked apoptosis occurred at all E:T ratios.
Peptide 10 μg/ml or OVA 40 μg/ml. In 96-well plates, 2 × 10^4 stimulated HA or OVA LNCs were then cocultured at a ratio of 1:10 with BALB/c splenocytes that had been infected overnight with the three-gene VVV, or with wt VV. Cells were cocultured for 5 days and pulsed for the last 18 h with ^3[H]TdR. ^3[H]TdR incorporated by HA cells cultured with the three-gene transduced APCs was reduced by >40%, as compared with control HA cells that had been cultured with wild-type VV-infected APCs (Fig. 4). OVA-specific T cells showed only minimal inhibition after coculture with the three-gene-transduced APCs. This experiment suggests that APCs with the three-gene VVV kill only the HA-specific T cells.

Ag targeting enhances FasL killing of HA-specific T cells

We compared the ability of APCs transduced with either the three-gene VVV or the VVV expressing FasL and TrFADD (but without the targeting construct HA-LAMP) to kill HA-specific T cells (Fig. 5). Target T cells from HA-transgenic mice were spleen cells that were first stimulated for 48 h with HA. APCs from BALB/c mice that had been infected overnight with either the three-gene VVV or the FasL + TrFADD VVV or control APCs infected with wt VV were added to the cultures and coincubated overnight. The cultures were harvested, and apoptosis was measured by standard ELISA (Boehringer Mannheim Cell Death Detection ELISA; Boehringer Mannheim, Indianapolis, IN). The background ODs were measured in supernatants from separately cultured APCs transduced with each of the VVVs. The background OD for APCs transduced with each specific VVV was subtracted from the results of the cocultures with the corresponding APCs, to give the ΔOD. The enrichment factor was calculated by dividing the ΔOD by the OD of HA-transgenic T cells that had been cultured alone (i.e., without APCs or Ag). The enrichment factor therefore represents apoptosis of T cells due to coculture with the particular VVV-transduced APCs. The results showed that HA-transgenic T cell cultures that were coincubated with three-gene VVV-transduced APCs exhibited marked apoptosis (enrichment factor = 2.43). By contrast, coincubation of HA-specific T cells with APCs that expressed FasL and TrFADD, but did not present HA, did not induce apoptosis (enrichment factor <1, which is not significant).

Time course of stimulation and killing of naive HA-specific T cells by APCs transduced with three-gene VVV

In preliminary experiments, we found that naive transgenic HA-specific T cells had to be stimulated for ~2 days to render them vulnerable to killing either by APCs transduced with the three-gene VVV or by Ab to Fas. This is consistent with the requirement for activation of T cells to induce up-regulation of Fas and vulnerability to FasL (9, 10, 22). To determine whether our transduced APCs could first stimulate, and then kill, these naive HA-specific T cells, we coincubated the T cells from transgenic HA mice with APCs that had been transduced with the three-gene VVV. Control APCs were infected with HA-LAMP VVV or wt VV. The cocultures with wt VV-infected APCs were either stimulated with 5 μg HA (as shown) or unstimulated and used for determination of backgrounds. Cocultures were grown for 8 days, and duplicate sets of cultures were pulsed with ^3[H]TdR for 18 h on each day from day 2 through day 8. The results indicated that all three groups were initially stimulated, reaching a peak on day 4 (Fig. 6). However, the T cells cocultured with three-gene APCs showed a rapid reduction of ^3[H]TdR incorporation after the peak and zero incorporation after day 5, suggestive of death of the cells. ^3[H]TdR incorporation declined slowly in the HA-stimulated T cells but remained active through the remainder of the 8-day experiment.

Discussion

Although current treatments of autoimmune diseases using general immunosuppressive agents are often effective, they have important drawbacks. The immune system as a whole is suppressed, thereby increasing the risks of infection and neoplasia, and the agents may have numerous other adverse side effects (23, 24). Ideally, treatment should eliminate the specific pathogenic autoimmune response, without otherwise suppressing the immune system. It
should have minimal or no other adverse side effects and should be long-lasting or permanent. The present study was designed to investigate and develop a novel strategy for specific immunotherapy, involving genetic engineering of APCs to target and eliminate Ag-specific T cells. Although conceptually simple, this ideal poses a number of difficult challenges. 1) All the relevant Ag-specific lymphocytes must be targeted. Because virtually all naturally occurring immune responses are not only highly heterogeneous but also unique to the individual (2–5), it is necessary to devise a method capable of targeting the entire spectrum of each individual’s specific T lymphocytes. The approach used in the present study utilizes the individual’s own APCs, genetically engineered to process and present the Ag of interest. These APCs can naturally target the entire repertoire of the same individual’s Ag-specific T cells, no matter how heterogeneous it may be. 2) The targeted T cells must be destroyed. For this purpose, we have provided the APCs with FasL as a warhead, which induces apoptosis of Fas-expressing T cells. 3) The APCs must be protected from self-destruction by the potentially dangerous FasL. We have used a gene that encodes a truncated mutant of FADD to protect against Fas-mediated apoptosis. 4) It is essential that all three gene constructs, i.e., for targeting the Ag, for expressing FasL, and for protecting the APCs, be inserted into and expressed simultaneously by the engineered APCs. Expression of one of these genes without the others could be counterproductive; Ag presentation alone could stimulate, rather than kill, the Ag-specific T cells; the lethal warhead alone, without the targeting system, might damage cells other than the Ag-specific T cells; FasL without the protective TrFADD could destroy the APCs. To assure simultaneous insertion of these gene constructs into APCs, we have developed a VVV that carries all three genes. 5) The vector must insert the genes only into the APCs; if other cells were to become infected, expression of the Ag or FasL could produce harmful results. To prevent such uncontrolled spread, we attenuate the VVV so that it does not replicate and spread, and we infect the APCs directly with VVV.

Our results clearly demonstrate that APCs expressing all 3 gene constructs induce apoptosis and death of HA-specific T cells, while sparing T cells with other specificities. Moreover, we have tested each component of this strategy and have shown that it works independently.

Ag presentation

“Professional” APCs normally process and present exogenously derived Ag (25, 26). However, endogenously synthesized proteins can also be processed and presented by APCs, provided that they are efficiently directed to the class II processing pathway. Recent studies have identified the protein signal LAMP-1, which can direct a variety of endogenously synthesized Ags to the class II pathways efficiently, resulting in greatly enhanced Ag presentation and T cell immune responses (6, 7, 27–29). This requires the LAMP-1 signal sequence at the 5’-terminus of the Ag to ensure translocation into the endoplasmic reticulum, as well as the transmembrane/cytoplasmic domain of LAMP-1 at its 3’-terminus (8, 30–32). Our results show that APCs transduced with recombinant VVV expressing the HA-LAMP-1 gene construct, alone or in combination with other genes, produced vigorous stimulation (and therefore targeting) of HA-specific T cells in vitro.

Fas-mediated cell death

The Fas-FasL system is of fundamental importance in the regulation of T lymphocytes. Fas (CD95) is present at the surface membranes of T cells, and is upregulated when they are activated (9, 10, 22). When FasL molecules interact with Fas, they cross-link Fas, initiating a series of steps that activate caspases and result in apoptosis of the Fas-bearing cells (33–35). In the present studies, we transferred the gene for FasL by means of recombinant VVV. Our results show that the FasL gene product is highly expressed by transduced cells, as demonstrated both by flow cytometry and functionally by killing stimulated HA-specific T cells as well as Fas-bearing A20 cells. Because of its lethal effect on activated T cells, FasL can therefore be used as a potent agent to eliminate Fas-expressing lymphocytes. Consistent with our findings, a previous study has shown that infection of APCs with adenovirus engineered to carry the gene for FasL induced T cell tolerance to the adenovirus, by producing apoptosis of T cells specific for the adenovirus, and infection of macrophages with FasL-expressing adenovirus induced tolerance to cell surface Ags of the macrophage (36, 37).

Truncated FADD

Studies of the role of FADD have led to a robust strategy for preventing Fas-mediated cell death. FADD is associated with the cytoplasmic portion of Fas and normally participates as an intermediary in the Fas-mediated cell death pathway. However, a FADD deletion mutant lacking aa 1–79 acts as a dominant negative, which inhibits Fas-mediated cell death (11, 12). We have obtained cDNA for this TrFADD mutant. Expression of the gene for TrFADD in the highly vulnerable A20 cells confers protection against cell death mediated by the Fas pathway (J.-M. Wu unpublished results). In the present study, we used VVV with the TrFADD gene in association with the gene for FasL, to avoid the risk of self-destruction of the APCs.

VV as a vector for gene transfer

VV has been used for >15 years as a vector for expression of genes in mammalian cells (14) including APCs (29). For our purposes, vaccinia has several important characteristics, including: 1) the ability to transfer multiple genes simultaneously; 2) high level production of the proteins encoded by the transferred genes; 3) the ability to be attenuated (by treatment with psoralen and UV light (16, 38)), so that it does not replicate (and therefore does not
specific immunotherapy by gene transfer to APC escape to infect the other cells) but does direct expression of the gene products by the infected cells.

Our results clearly demonstrate the validity of the principles embodied in the “guided missile” strategy. We have shown that APCs genetically engineered to process and present epitopes of a model Ag and to express FasL can target and induce apoptosis of Ag-specific T cells. Our results demonstrated the specificity of the lethal effect of Ag-directed APCs. Thus, OVA-specific T cells were not affected significantly by three-gene APCs that presented HA-specific epitopes, even though the T cells were appropriately stimulated and in close physical contact with the APCs. Intimacy of contact between T cells expressing Fas and APCs expressing FasL appears to be necessary for induction of apoptosis. APCs expressing the two gene products, FasL and TrFADD, but not presenting HA did not induce apoptosis of HA-specific T cells. This is consistent with reports that FasL-expressing cells must bind their Fas-expressing victims to induce apoptosis (39, 40). Furthermore, for T cells to undergo Fas-mediated apoptosis, they must be activated and must express Fas strongly. Our findings showed that naive HA-transgenic T cells required Ag stimulation in order to be susceptible to apoptosis induced by the FasL-expressing APCs. The time course experiment showed that the three-gene APCs were able to kill naive HA-specific T cells in culture but that they first stimulated the T cells before inducing apoptosis.

The lethal (or inhibitory) effect of the three-gene-transduced APCs could not be attributed to Ag-induced cell death, but rather to the effect of the FasL expressed by transduction of the APCs. Parallel experiments in which the HA-specific cells were confronted with APCs that were transduced with the stimulatory HA-LAMP-1 VVV and the three-gene VVV showed that stimulation per se did not induce apoptosis of the targeted cells whereas Ag targeting and expression of FasL effectively induced apoptosis.

Certain features of these studies require comment. First, as a convenient source of abundant and highly reproducible Ag-specific T cells, we used a well-defined model murine system, with transgenic T cells that express the αβTCR for HA. Although the Ag-specific T cells used here were actually monoclonal, we have also had equally striking success with a heterogeneous Ag-specific T cell population (41). Second, for the purposes of the present experiments, we have used the VV for gene transfer. The advantages of vaccinia for this purpose have been stated, but other methods of gene transfer may prove useful in the future. The use of retroviral vectors that can express multiple foreign genes is under investigation in our laboratories. Third, as a convenient source of APCs, we have used a mixed population of spleen cells, including macrophages, dendritic cells, and B cells. Now that new methods of obtaining relatively large populations of dendritic cells are becoming available, we are evaluating the feasibility of using dendritic cells as APCs in these experiments. Ultimately, it should be possible to develop the guided missile strategy described here for the treatment of autoimmune diseases.

Acknowledgments
We are grateful for helpful discussions with Dr. Drew Pardoll.

References
1. Hohlfeld, R., and H. Wekerle. 1998. Novel therapeutic approaches to autoimmu-


