Cutting Edge: Restoration of the Ability to Generate CTL in Mice Immune to Adenovirus by Delivery of Virus in a Collagen-Based Matrix

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Viruses are commonly used for the delivery of genes coding for tumor-associated Ags to elicit tumor-specific immune responses. The success of viral vectors has been limited in preclinical and clinical trials in part because of antiviral immunity. We investigated the ability of a collagen-based matrix (Gelfoam; Pharmacia and Upjohn, Kalamazoo, MI) to improve CTL activation by recombinant adenovirus. The data show that coinjection of Gelfoam with type 5 adenovirus recombinant for prostate-specific Ag (Ad5-PSA) enhanced CTL activation. Ad5-PSA priming in Gelfoam also abrogated the inhibitory effects of adenoviral immunity on CTL activation in mice naive to PSA but immune to adenovirus. Finally, Gelfoam enhanced immunization in a self-Ag model using type 5 adenovirus recombinant for membrane-bound OVA (Ad5-mOVA) in rat insulin promoter (RIP)-mOVA-transgenic mice. Thus, Gelfoam enhances CTL activation by recombinant viral vectors in a setting where preformed Ab to the virus is present and also in a tolerant self-Ag model. The Journal of Immunology, 2001, 166: 731–735.

An important objective of immunotherapy is the activation of CTL responses to tumor-associated Ags. Considerable effort has focused on the identification of relevant T cell Ags, yielding an array of Ags ranging from those unique to tumors to those shared by tumor cells and parenchymal cells of similar origin (1–7). One of the primary means of activating immunity to identified Ags is through the use of recombinant technology. Genes coding for the relevant Ag are incorporated into delivery vectors, commonly viruses, which have proven to be highly effective in generating transgene expression and in CTL activation.

A number of investigators have reported effective CTL generation with recombinant viral vectors that effectively controlled tumor growth (8–11). Although viruses are effective in generating CTL-mediated antitumor activity in naive mice, the use of viruses in settings where the host has been previously exposed to the virus significantly reduces transgene expression (12, 13). Studies by Yang and associates formally demonstrated that T cell responses, specifically CTL, to viral proteins were responsible for destruction of cells expressing the transgene (12). Furthermore, Abs in the serum diminish the ability of viruses to deliver the transgene (13), and the resulting decrease in gene expression reduces subsequent CTL activation (14–17). The diminished ability to generate CTL, when Abs to the viral vector are present, has been suggested to be an important reason for the lack of CTL after adenovirus delivery of melanoma Ags in clinical trials (18).

Numerous attempts to circumvent the adverse effects of viral immunity on gene expression and immune activation have been reported, including boosting regimens with a different viral vector (19) and inhibiting the host anti-viral immune response with agents such as soluble TNF receptor (20), CTLA4Ig (21), or cyclophosphamide (22). The collagen-based matrix, Gelfoam (Pharmacia and Upjohn, Kalamazoo, MI) is primarily used as an intraoperative hemostatic agent, but also has been used to deliver a number of different compounds, including insulin (23), various cytokines, and growth factors (24, 25), to improve and sustain delivery. We have recently reported the use of Gelfoam as a solid-state delivery vehicle that significantly enhanced subsequent gene expression and improved tumor inhibition in an in situ immunotherapy protocol (26). Here, we demonstrate the ability of Gelfoam to enhance the generation of CTL, both in the presence of neutralizing Abs to the delivery virus as well as in a self-Ag model.

Materials and Methods

Animals and tumor cells

The BALB/c murine prostate cancer model, RM-11, was obtained from Dr. Timothy C. Thompson (Baylor College of Medicine, Houston, TX) (27). A
Gene transfer vectors

A replication-deficient type 5 adenovirus recombinant for PSA (Ad5-PSA) expressed from the CMV promoter was generated using standard methods by the University of Iowa Gene Transfer Vector Core (Iowa City, IA) (30). Briefly, the entire coding sequence of PSA cloned into pAd5CMVK-NpA. The resultant plasmid and adenovirus backbone sequences that had the E1 (E1A and E1B) genes deleted were transfected into human embryonic kidney (HEK) 293 cells, and viral particles were isolated and amplified for analysis of PSA expression. Type 5 adenovirus recombinant for OVA (Ad5-mOVA) used for experiments in the self-Ag model, as well as the control vector Ad5-lacZ (31), were prepared as described for Ad5-PSA.

Delivery system and immunizations

Gelfoam is a collagen matrix prepared from purified pork skin collagen (23–26). Preparation of the viral vectors in the Gelfoam for injections was performed as previously described (26). The matrix/virion mixture was injected s.c. into the flanks of mice. CTL activity induced by matrix-delivered vectors was compared with s.c. injection of the same number of viral particles without matrix (fluid phase).

To investigate possible benefit of Ad5-PSA immunization with Gelfoam in the presence of anti-adenovirus Abs, mice were injected i.p. with Ad5-lacZ (10^9 PFU) 2 wk before immunization with Ad5-PSA. Control mice were injected i.p. with PBS. Serum samples were obtained before and after immunization and subsequently assayed for adenovirus-specific Ab titers.

Isolation and detection of lytic T cells

Fourteen days after virus injection, splenocytes were isolated and tested for CTL activity as previously described (17). Briefly, for PSA-specific CTL, splenocytes were incubated in 24-well plates at 1 × 10^7 cells/well together with the mitomycin C-treated P815psa stimulator cells (4 × 10^4) in DMEM supplemented with 10% FCS, 5 × 10^{-5} M 2-ME, and 10 IU/ml recombinant human (rh)IL-2. The CTL assays in the RIP-mOVA self-Ag model were performed in a similar fashion, except that in vitro stimulation was performed as a 5-day coculture with Ad5-mOVA-infected dendritic cells or SIMFELK-pulsed 1500 Gy irradiated syngeneic spleen cells as stimulators (17, 32, 33). CD8-depleted effector cells were obtained by depleting CD8^+ T cells after the 5-day culture with the Vario Macs magnetic bead system (Miltenyi Biotec, Auburn, CA) as described by the manufacturer. CD8-depleted effector cells contained <2% CD8^+ T cells as determined by flow cytometry.

Results

Effect of matrix on CTL activation

Previous studies demonstrated that Gelfoam enhanced the distribution and expression of genes delivered by viral vectors in vivo (26). To determine whether Gelfoam also enhanced CTL activation, Ad5-PSA (1 × 10^7–1 × 10^9 PFU) was injected into BALB/c mice with and without Gelfoam. Injection of mice with Ad5-lacZ (1 × 10^7 PFU) served as a negative control. Detection of CTL against PSA-expressing targets was observed without Gelfoam only at the highest virion concentration (1 × 10^9 PFU; Fig. 1A). Immunization with Gelfoam consistently demonstrated significant specific lysis at lower virion concentrations (1 × 10^7–1 × 10^9 PFU, Fig. 1B) in at least three separate experiments. Further studies in which either CD8^+ T cells were depleted with anti-CD8 mAb (clone 2.43) or anti-CD8 was added to the lytic assay resulted in abrogation of lysis, demonstrating the effector cell population to be CD8^+ T cells (data not shown).

Restoration of CTL activation in adenovirus-presensitized mice

Previous studies showed diminished CTL activity to transgene products if delivered by a viral vector to which the animal was immune (14–17). Because the impact of viral immunity on CTL activation has been implicated as a negative factor in clinical studies and Gelfoam showed the capacity to enhance CTL activity, we tested the effect of Ad5-PSA in Gelfoam on CTL activation in the presence of anti-adenoviral immunity (18). Mice were first injected i.p. with 1 × 10^9 PFU Ad5-lacZ or a similar volume of PBS. Serum anti-adenoviral Ab titers of Ad5-lacZ-injected mice from both Ad5-PSA and Ad5-PSA with Gelfoam groups showed high levels of anti-adenovirus Ab at the time of Ad5-PSA injection (52.4 and 62.1 μg/ml, respectively). Subsequently, Ad5-lacZ-primed and control mice were immunized with Ad5-PSA with and without Gelfoam. As expected, prior infection with Ad5-lacZ significantly inhibited the ability of Ad5-PSA to induce a PSA-specific CTL response (Fig. 2). However, immunization with the same Ad5-PSA concentration (1 × 10^9 PFU) delivered with Gelfoam restored PSA-specific CTL lytic activity to the levels observed in adenovirus-naive mice.

CTL in a self-Ag model

Significant improvement of immunization was demonstrated using Gelfoam as a delivery vehicle in the foreign Ag model using human PSA in a murine system, which lacks a PSA homolog. However, limited success in activating CTL has been reported in nonmutated self-Ag models (9). To determine whether viral delivery
with Gelfoam would improve immunization to self-Ags, we used RIP-mOVA mice, which develop tolerance to OVA-specific peptides (29, 32). RIP-mOVA mice were immunized with Ad5-mOVA (1 x 10^8 PFU) in the fluid phase with and without Gelfoam. Ad5-mOVA (1 x 10^8 PFU) in the fluid phase also was injected into both RIP-mOVA mice and, as a control, into normal C57BL/6 mice. Immunization of normal C57BL/6 mice demonstrated OVA-specific lysis of EG.7 target cells, whereas Ad5-mOVA immunization (i.p. or s.c.) of RIP-mOVA mice showed no OVA-specific lysis (Fig. 3).

In contrast, Ad5-mOVA immunization in Gelfoam stimulated significant OVA-specific CTL. Lysis by cells activated with Ad5-mOVA in Gelfoam was mediated by CD8^+ T cells (Fig. 3, inset). Moreover, histological evaluation of the pancreas from Ad5-mOVA-plus-Gelfoam-primed mice showed β-islet-associated inflammation but no obvious β-islet destruction (data not shown).

**Discussion**

Since the first convincing demonstration of the existence of specific tumor-associated Ags (34), much interest has been stimulated in the possibilities of cancer immunotherapy. Recently, numerous reports have identified genes encoding Ags specific to tumor cells, suggesting that recombinant tumor Ags could be delivered to the host to elicit an anti-tumor immune response. Commonly, these engineered peptides or proteins have been delivered to the host as a recombinant viral vaccine using vaccinia or adenovirus. Despite the benefits of transferring genetic material in a viral vector, the presence of preexisting immunity or the development of a neutralizing immune response to viral proteins after treatment limits the use of these vectors as initial immunizing agents or for readministration to boost the response (12–18).

Several strategies have been adopted to overcome or bypass this neutralizing immune response to optimize the use of these viral vectors. Tumor Ags can be delivered as defined peptides (35), proteins designed to access the MHC class I pathway of APCs in vivo (36), or by adoptive transfer of Ag-loaded APCs such as dendritic cells (37). DNA vaccines encoding tumor Ags can be delivered as naked DNA or encapsulated by liposomes (38). Xiang et al. have recently described an autologous oral DNA vaccine delivered with an attenuated strain of Salmonella typhimurium to successfully immune mice in a self-Ag model of melanoma (39).

In addition, less immunogenic vectors have been used including adeno-associated virus, lentivirus, and gutless adenovirus. Also, avoidance of the antiviral immune response of the more immunogenic viral vectors may be possible by varying the vectors that are used for boosting the immune response (19). Finally, treatment with suppressive agents such as soluble TNF receptor (20), CTLA4Ig (21), cyclophosphamide (22), and anti-CD40 (40) has been shown to inhibit the host anti-viral immune response, improve transgene expression, and enhance the ability to use vectors for multiple injections. Although the latter suppressive approaches are effective in blocking the development of immunity to viral proteins, their use in the context of vector-induced CTL activation...
is controversial. Clearly, alternative approaches for gene delivery and CTL activation are needed.

Our previous data demonstrated that intratumoral injection of viral vectors in Gelfoam enhanced both the distribution and the expression level of reporter transgenes transferred by viruses (26). Furthermore, the enhanced gene delivery with the Gelfoam enhanced biologic activity of an in situ immunotransfer protocol (26). As a result of the enhanced gene delivery and augmented antitumor effects induced by coinjection of Gelfoam and recombinant viruses, we tested the effects of Gelfoam on Ad5-PSA-mediated CTL activation. Ad5-PSA was previously observed to induce CTL activity in a murine tumor model system, and to activate CTL-mediated PSA-specific antitumor activity.3 Here we show that Gelfoam enhances CD8+ CTL activation in both foreign and autologous Ag settings. Consistent with previous reports in the RIP-mOVA model where OT-1 adoptive transfer frequency was low, in the autologous Ag settings. Consistent with previous reports in the RIP-mOVA model where OT-1 adoptive transfer frequency was low, inflammation was observed in the pancreas but β-islet destruction was not apparent (Ref. 40, and data not shown). Importantly, we show that administration of Ad5-PSA with Gelfoam abrogated the inhibitory effects on CTL activation of preexisting immunity to adenvirus. These data suggest that coinjection of recombinant viruses with Gelfoam may provide an approach for augmenting CTL activation in the clinical setting and also may provide a means of using viral vectors for multiple injections to boost immunity.

The mechanism(s) by which Gelfoam enhances CTL priming is not known. Previous studies demonstrated that CTL priming either with pox or pox virus required MHC class I-matched bone marrow-derived cells. Interestingly, either recombinant Ag produced in the periphery or Ag produced by bone marrow-derived cells was sufficient to activate CTL (41). In this regard, distribution studies, which were performed using PCR amplification of adenoviral DNA, showed an absence of detectable DNA in the liver, lungs, and other tested organs, but adenoviral DNA was detected in the draining lymph nodes for both fluid phase and Gelfoam-delivered virus. These initial biodistribution studies were not quantitative but clearly show the presence of virus in draining lymph nodes. Perhaps Gelfoam enhances delivery to the draining nodes where higher Ag expression could enhance T cell priming (42). It is clear that the delivery of genes in Gelfoam results in a 5- to 10-fold enhancement of gene expression and an extension of the time course of expression (26). Likewise, it is known that Ag levels play an important role in CTL priming (33, 43). In this regard, Xiang and associates recently showed that by directing proteins to the protosome, which enhanced MHC class I Ag presentation and limited Ab development, CTL activation was enhanced sufficiently to mediate antitumor activity (39). Thus, the simplest explanation for the observed ability of Gelfoam to augment CTL activation is elevated production of the transgene product, which delivers a higher Ag load to bone marrow-derived APCs. Determination of the functional role of Gelfoam is currently under investigation.

References


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