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Dendritic Cell Maturation, but Not CD8⁺ T Cell Induction, Is Dependent on Type I IFN Signaling during Vaccination with Adenovirus Vectors

Scott E. Hensley,*† Wynetta Giles-Davis,† Kimberly C. McCoy,† Wolfgang Weninger,† and Hildegund C. J. Ertl²**†

To understand how vaccines initiate adaptive immune responses, it is necessary to study how they interact with APCs such as dendritic cells (DCs). In this study, we analyzed interactions between recombinant adenovirus (Ad) vectors and mouse DCs. Mouse bone marrow-derived DCs transduced with Ad vectors produced type I IFN, which promoted the maturation of both transduced and bystander DCs. DCs transduced with a vector derived from a chimpanzee Ad serotype (AdC68) produced more type I IFN and matured more efficiently compared with DCs transduced with a vector derived from a human Ad serotype (AdHu5). Both vectors stimulated type I IFN production independently of viral transcription, replication, and TLR signaling. However, each vector induced type I IFN through distinct pathways; whereas AdHu5 vectors required phosphoinositide-3-OH kinase for type I IFN induction, AdC68 vectors did not. Both vectors induced strong transgene product-specific CD8⁺ T cell responses in wild-type mice. DCs isolated from mice that have a defect in type I IFN signaling failed to undergo full maturation after Ad vaccination, but surprisingly, these mice mounted strong transgene product-specific CD8⁺ T cell responses. In these mice, we were able to detect a small number of transduced DCs that expressed high levels of costimulatory molecules, and these DCs were able to stimulate transgene product-specific CD8⁺ T cells. Thus, type I IFN signaling is an important component of Ad-mediated DC maturation but is dispensable during the generation of transgene product-specific CD8⁺ T cell responses. The Journal of Immunology, 2005, 175: 6032–6041.

Recombinant adenovirus (Ad)³ vectors elicit potent transgene product-specific CD8⁺ T and B cell responses (1). Most preclinical and clinical studies have examined the vaccine efficiency of vectors derived from common human Ad serotypes such as AdHu5 and AdHu2. To circumvent problems associated with pre-existing immunity to common human Ads, recent studies have used vectors derived from uncommon human Ads (2–4) or Ads that naturally infect species other than humans (5, 6). We have shown that a recombinant vector derived from a chimpanzee Ad, termed “AdC68,” elicits strong transgene product-specific CD8⁺ T cell responses in mice that are pre-exposed to AdHu5 (7). Furthermore, prime-boost regimens using heterologous recombinant chimpanzee Ad vectors result in the production of high levels of transgene product-specific CD8⁺ T cells in non-human primates (8).

Induction of primary adaptive immune responses requires presentation of Ag by professional APCs such as dendritic cells (DCs) (9). When given the appropriate stimulus, immature DCs in the periphery undergo a maturation process characterized by the up-regulation of costimulatory molecules and secretion of proinflammatory cytokines. During this process, DCs up-regulate chemokine receptors such as CCR7, leading to their migration to secondary lymphatic organs where they present Ag to T cells. Ad vectors efficiently transduce and express transgene in DCs (10–12); however, viral gene expression is not necessary for Ad-mediated DC maturation (12–14). Although capsid components of Ad vectors are thought to trigger DC maturation, the exact viral proteins involved remain controversial (15–17).

The binding of pathogen-associated molecular patterns to pattern recognition receptors (PRRs) expressed on or within DCs triggers DC maturation. The best-characterized group of PRRs is TLRs, a group of 11 different receptors that recognize a variety of pathogens (18). Viral components such as dsRNA, ssRNA, and viral DNA can activate TLR pathways (19–24). Other TLRs recognize viral coat proteins from measles virus (25), respiratory syncytial virus (26), and murine retroviruses (27). Activation of PRR by some pathogens leads to the production of type I IFNs (28–30). This group of pleiotropic cytokines promotes the induction of CD8⁺ T cells during certain viral infections (31) and is an important signaling component during LPS and poly(I:C)-mediated macrophage (32) and DC maturation (33). Some viruses, such as Newcastle disease virus, HSV type I, and lymphocytic choriomeningitis virus induce DC maturation through pathways that are dependent on type I IFN signaling (33–35), whereas other viruses such as Sindbis virus induce DC maturation through pathways independent of type I IFN signaling (36).

In this study, we investigated whether type I IFN signaling is involved in Ad-mediated DC maturation and whether it is essential for the induction of transgene product-specific CD8⁺ T cells following Ad vector immunization. We found that DCs transduced

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with AdHu5 or AdC68 vectors produce type I IFN, and that type I IFN signaling is a critical component of Ad-mediated DC maturation. DCs transduced with AdC68 vectors produce more type I IFN than DCs transduced with AdHu5 vectors, and subsequently undergo maturation more efficiently. Our results indicate that Ad-induced type I IFN production does not require TLR signaling or viral replication. DCs from mice with a defect in type I IFN signaling have an impaired ability to undergo maturation after Ad vaccination. However, a small number of transduced DCs that express high levels of costimulatory molecules can be found in these mice after Ad vaccination, and these DCs are able to prime transgene product-specific CD8+ T cell responses.

Materials and Methods

Mice

129Sv/Ev mice were obtained from Taconic, C57B/6 mice were obtained from Charles River Laboratories, OT1 mice (C57B/6 background) were obtained from The Jackson Laboratory, C3H/HeN and C3H/HeJ mice were obtained from The Jackson Laboratory, C3H/HeJ mice (C57B/6 background) (37) were obtained from L. Buxbaum (University of Pennsylvania, Philadelphia, PA). MyD88−/− mice (C57B/6 background) (37) were obtained from S. Ross (University of Pennsylvania, Philadelphia, PA) with permission from S. Akira (Osaka University, Osaka, Japan), and TLR3−/− mice (C57B/6 background) (19) were obtained from R. Flavell (Yale University, New Haven, CT). Mice were used at 5–7 wk of age, and all animal studies were performed according to institutional approved protocols.

Vectors

The NP.S.EGFP cassette (38) was kindly provided by J. Yewdell (National Institutes of Health, Bethesda, MD) and cloned into a shuttle vector and molecular biology techniques. These vectors, as well as Ad vectors expressing only EGFP, were propagated on HEK 293 cells and purified by CsCl gradient centrifugation as previously described (5, 39). Particle number was determined by Odsol readings, and infectious units were determined by PCR. Aliquots of vector were inactivated by treatment with 8-methoxypsoralen (Sigma-Aldrich) and 360-nm UV irradiation for 60 min using a protocol previously described (40). All vectors were determined to be free of endotoxin using a QCL-1000 Chromogenic LAL Test kit (Cambrex Bioscience).

Bone marrow-derived DC (BM-DC) preparation

BM-DCs were prepared as previously described (41). BM-DCs were grown in medium containing 20 ng/ml GM-CSF (Peprotech), harvested at day 10, and reseeded at 2.5 × 105 cells/well in 96-well plates. Vector (dosed by particle number), LPS (Sigma-Aldrich), or poly(I:C) (Amersham Biosciences) was added to the BM-DCs in complete medium containing 10 ng/ml GM-CSF immediately after reseeding the cells, and supernatant was collected 24 h later. In some experiments, BM-DCs were preincubated with wortmannin (Sigma-Aldrich) for 1 h at 37°C. IFN-α levels in supernatants were determined by ELISA using a previously described protocol (42).

DC isolation from lymph nodes

Mice were vaccinated i.m. in the lower leg, and the popliteal and inguinal lymph nodes were removed 24 h later. Single-cell suspensions were prepared and treated with collagenase (Liberase Blendzymes; Roche) and DNase I (Roche) for 15 min at 37°C and then with EDTA for 5 min at room temperature. The cells were then passed through a 70-μm nylon cell strainer (BD Falcon) and washed twice with L-15 (Media-tec).

DC stimulatory molecule expression

BM-DCs or cells isolated from lymph nodes were plated in 96-well plates and incubated with purified anti-mouse CD16/CD32 for 30 min at 4°C. Cells were then incubated for 30 min at 4°C with alkaline phosphatase-labeled anti-mouse CD11c and PE-labeled anti-mouse CD86, CD80, CD40, H-2Kb, or I-A, or I-Aβ. Some cells were incubated with alkaline phosphatase-labeled anti-mouse CD11c and biotinylated 25D1.16, which is specific for H-2Kb (BioLegend, San Diego, CA), and Alexa Fluor 488-labeled anti-CD86, CD80, CD40, or H-2Kb (eBioscience, San Diego, CA). For some experiments, PE-labeled anti-CD86, CD80, or CD40, or H-2Kb (eBioscience, San Diego, CA) was used, and these samples were analyzed using Cyan-LX. Unless specified, all Abs were purchased from BD Pharmingen. Flow cytometry for this set of experiments was performed using CYAN-LX (DakoCytomation).

T cell proliferation assay

CD11c+high cells from popliteal and inguinal lymph nodes were sorted using a DakoCytomation MoFlo (DakoCytomation) and plated in RPMI 1640 medium in 96-well round-bottom plates. CD8+ cells were isolated from the spleens of OT1 mice by negative selection using magnetic beads (Miltenyi Biotec), and 5 × 104 of these cells were transferred to each well. Three days later, cultures were pulsed for 16 h with 1 μCi of [3H]thymidine (PerkinElmer/well) and [3H]thymidine incorporation was measured using a Matrix 96 β counter (Packard) after transferring cells to a glass fiber filter (Packard).

Intracellular cytokine staining

Mice were vaccinated i.m. in the lower leg with 1 × 106 particles/mouse AdHu5-NP.S.EGFP or AdC68-NP.S.EGFP and sacrificed at the indicated time points. Splenocytes were cultured for 5 h at 37°C with brefeldin A (BD Pharamingen) and SINFEKL peptide (Alpha Diagnostic International) or ASNENMDM peptide (gift from W. Gerhard (The Wistar Institute, Philadelphia, PA)). Control samples were incubated with an unrelated peptide (AMQLKETI) from the gag protein of HIV-1. For most experiments, 100 ng/ml peptide was used for stimulation; however, a wide range of peptide concentrations was used for avidity studies. After incubating with peptide and brefeldin A, cells were washed and incubated with FITC-labeled anti-mouse CD8 for 30 min at 4°C. Cells were permeabilized in 1× Cytofix/Cytoperm (BD Pharamingen) for 20 min at 4°C, washed with Perm/Wash (BD Pharamingen), and incubated with PE-labeled anti-mouse IFN-γ. Cells were washed again and analyzed by flow cytometry using an EPICS Elite XL (Beckman Coulter). For some samples, PE-labeled anti-mouse CD8, FITC-labeled anti-mouse IFN-γ, and allophycocyanin-labeled anti-mouse TNF-α were used, and these samples were analyzed using Cyan-LX (DakoCytomation). For other samples, PE-labeled anti-mouse CD8, FITC-labeled anti-mouse CD107a/b, and allophycocyanin-labeled anti-mouse IFN-γ were used, and these samples were analyzed using Cyan-LX. Unless specified, all Abs were purchased from BD Pharmingen.

Statistical analysis

Student’s t tests and ANOVA Bonferroni/Dunn posthoc tests were performed using MATLAB software.

Results

Ad-transduced DCs produce type I IFN which promotes DC maturation

We generated Ad vectors that express a fusion protein composed of the nucleoprotein (NP) of influenza A virus (containing the H-2Dβ binding epitope ASNENMDM), the H-2Kb binding epitope SINFEKL from OVA, and enhanced GFP (EGFP). Using vectors expressing this cassette (NP.S.EGFP), we were able to measure SINFEKL- and ASNENMDM-specific CD8+ T cell responses as well as GFP levels in Ad-transduced cells. The particle/infectious units (pt/IU) ratio of the vectors was 29.5 pt/IU for AdC68.NP.S.EGFP and 12.2 pt/IU for AdHu5.NP.S.EGFP. All vector doses used in this study were based on particle number.

We have previously shown that mouse splenocytes transduced with Ad vectors produce high levels of type I IFNs (44), and Ad vector transduction of human DCs results in IFN-α production (45). To determine whether type I IFN signaling is necessary for Ad-mediated DC maturation, BM-DCs were generated from healthy mice or mice lacking the IFN-α/β receptor component IFNAR1 (IFNAR−/−) were transduced with either AdHu5 or AdC68 vectors. BM-DCs from IFNAR−/− mice did not produce IFN-α after transduction with AdHu5 or AdC68 vectors, indicating that Ad-induced IFN-α production is augmented through an autocrine pathway.

Transgene product levels within transduced BM-DCs inversely correlated with IFN-α production (Fig. 1B, Table I). BM-DCs transduced with the AdHu5 vector expressed higher levels of GFP.
compared with BM-DCs transduced with the AdC68 vector. Although AdC68 and AdHu5 vectors have extensive sequence homology, there are differences in the fiber and penton proteins that may affect entry into cell types such as DCs. BM-DCs transduced with the AdHu5 vector also express higher levels of MHC class I/SHIFELK complexes on the cell surface compared with DCs transduced with an equal dose of the AdC68 vector (Fig. 1C). Therefore, the disparity in GFP expression likely reflects differences in vector uptake and/or protein synthesis rather than accelerated degradation of protein.

BM-DCs isolated from 129Sv/Ev mice expressed elevated levels of CD86, CD80, CD40, MHC class II, and MHC class I after transduction with Ad vectors, whereas BM-DCs isolated from IFNAR−/− mice did not (Fig. 2A). AdC68 vectors, which induced higher levels of type I IFN compared with AdHu5 vectors, were more efficient in driving maturation of 129Sv/Ev BM-DCs. High doses (10,000 particles/cell) of either vector strongly induced maturation of 129Sv/Ev BM-DCs but not IFNAR−/− BM-DCs (data not shown). Ad-induced up-regulation of costimulatory molecules on 129Sv/Ev BM-DCs did not require direct transduction (Fig. 2B). Thus, in addition to activating DCs in an autocrine manner, Ad-induced type I IFN also activates bystander DCs. Consistent with previous reports (33), IFNAR−/− BM-DCs partially up-regulated costimulatory molecules when exposed to LPS but did not mature when exposed to poly(I:C) (Fig. 2C). Therefore, IFNAR−/− BM-DCs can mature when given the appropriate stimulus but fail to do so after transduction with Ad vectors.

Ad-induced type I IFN does not require viral transcription, viral translation, or TLR signaling

During viral infections, dsRNA often triggers type I IFN production. The Ad vectors in this study were replication-defective; however, such vectors can express low levels of viral transcripts, and a byproduct of Ad transcription is dsRNA (46). The induction of IFN-α by Ad vectors occurs independently of viral replication and/or transcription as shown by IFN-α levels produced by BM-DCs transduced with UV-inactivated Ad vectors (Fig. 3A). UV-inactivated and untreated AdC68 vectors induced similar levels of IFN-α. UV-inactivated AdHu5 vectors induced higher levels of IFN-α compared with untreated AdHu5 vectors, indicating that AdHu5 vectors may produce a gene product that inhibits IFN-α production. In agreement with previous reports (12–14), UV-inactivated Ad vectors induced DC maturation, as determined by the up-regulation of CD86 (Fig. 3B). The UV-inactivated vectors did not express GFP, indicating that they were fully inactivated (Fig. 3B).

TLRs recognize diverse pathogens and initiate signaling events that lead to the production of cytokines such as type I IFN (18). All TLRs are capable of signaling through the adaptor molecule MyD88, which has been shown to be nonessential for AdHu5 vector-induced DC maturation (17). Some TLRs, such as TLR3 and TLR4, can also signal independently of MyD88 (18). To determine whether Ad induces type I IFN production through TLR-dependent pathways, BM-DCs from mice with defects in TLR signaling were transduced with Ad vectors. MyD88−/−, TLR3−/−, and C3H/HeJ (which have a natural occurring TLR4 mutation) BM-DCs produced levels of IFN-α comparable to that of wild-type BM-DCs (Fig. 3, C and D) and showed similar levels of costimulatory molecule up-regulation (data not shown). Thus, Ad-induced IFN-α production and DC maturation does not depend on known TLR signaling cascades.

Chimpanzee Ad vectors and human Ad vectors induce type I IFN through different pathways

AdHu5 and AdC68 vectors attach to the surface of most cell types through high-affinity binding of fiber protein to the coxsackie and Ad receptor (47, 48), and subsequent endocytosis of Ad is promoted by penton binding to α,β integrins (49). Murine DCs do not express high levels of coxsackie and Ad receptor (12, 16, 50) but do express α,β integrins, and it is thought that Ad vectors enter...
DCs through a process that relies on penton binding to $\alpha_v\beta_3$ integrins.

AdHu5 penton interactions with integrins stimulate phosphoinositide-3-OH kinase in cell lines (51), and DCs transduced with AdHu5 vectors produce TNF-$\alpha$ through a phosphoinositide-3-OH kinase-dependent pathway (17). TNF-$\alpha$ is essential for AdHu5-induced DC maturation and a chemical inhibitor of phosphoinositide-3-OH kinase, wortmannin, blocks DC maturation induced by AdHu5 vectors (17). To test whether phosphoinositide-3-OH kinase is necessary during Ad-induced IFN-$\gamma$ production, we transduced BM-DCs with AdHu5 or AdC68 vectors in the presence of wortmannin. The addition of wortmannin decreased AdHu5 vector induced IFN-$\gamma$ in a dose-dependent manner, but had no effect on AdC68 vector-induced IFN-$\gamma$ production (Fig. 4A). Correspondingly, wortmannin blocked AdHu5 vector-induced DC maturation but had no effect on AdC68 vector-induced DC maturation (Fig. 4B). This suggests that AdC68 and AdHu5 vectors induce IFN-$\gamma$ through distinct pathways. AdHu5 vector-induced IFN-$\gamma$ production likely requires a penton interaction with an $\alpha_v\beta_3$ integrin, whereas AdC68 vector-induced IFN-$\gamma$ production does not require this interaction.

Type I IFN signaling is critical for driving full DC maturation in vivo but not for the generation of transgene product-specific CD8$^+$ T cells after vaccination with Ad vectors

The generation of adaptive immune responses is dependent on Ag presentation by professional APCs and requires interactions between Ag-specific T cell receptors and MHC peptide complexes, as well as a second signal provided by costimulatory molecules on APCs. The major type of DCs that Ad vectors transduce in vivo are myeloid DCs, and these cells are the most effective APCs during vaccination with either AdHu5 or AdC68 vectors (S. E. Hensley and H. C. J. Ertl, unpublished data). To study changes in costimulatory molecule expression on DCs in vivo, we vaccinated 129Sv/Ev or IFNAR$^{-/-}$ mice with Ad vectors i.m. and analyzed CD86 expression on myeloid DCs (which express high levels of CD11c). We chose to administer the vector i.m., because this route of vaccination leads to the generation of higher levels of transgene product-specific CD8$^+$ T cells compared with other routes of vaccination such as oral or intranasal vaccination (52, 53). Twenty-four hours after vaccination, the draining lymph nodes and the spleens were removed from the vaccinated mice and single-cell suspensions were stained with Abs against CD11c and CD86. In 129Sv/Ev mice, CD11c$^+$ DCs expressed elevated levels of CD86 after injection of Ad vectors, and the levels of these molecules were higher on DCs after injection with AdC68 vectors compared with injection with AdHu5 vectors (Fig. 5). There was a slight increase of CD86 expression on CD11c$^{\text{high}}$ DCs isolated from the lymph nodes of Ad vector-injected IFNAR$^{-/-}$ mice, but this increase was minimal compared with the increase found in 129Sv/Ev mice. Splenic CD11c$^{\text{high}}$ DCs from vaccinated 129Sv/Ev mice expressed elevated levels of CD86. This did not occur to the same extent in vaccinated IFNAR$^{-/-}$ mice. This indicates that Ad-induced type I IFN also promotes the activation of bystander DCs in vivo, even in lymphatic tissues distant from the site of vector injection. In vaccinated 129Sv/Ev mice, an up-regulation of CD86 was observed when gating on total live cells (which includes cell types other than DCs), but this did not occur in vaccinated IFNAR$^{-/-}$ mice.
To determine whether type I IFN-dependent DC maturation affected the activation of transgene product-specific CD8+ T cells, we measured the frequency of SIINFEKL-specific CD8+ T cells in 129Sv/Ev or IFNAR−/− mice vaccinated with Ad vectors expressing NP.S.EGFP. Splenocytes were tested for SIINFEKL-specific CD8+ T cells by intracellular cytokine staining for IFN-γ at various times after vaccination. 129Sv/Ev mice mounted a strong SIINFEKL-specific CD8+ T cell response after injection of AdC68 or AdHu5

FIGURE 3. Ad-induced IFN-α production does not require viral transcription, translation, or TLR signaling. A, 129Sv/Ev BM-DCs were transduced with either UV-inactivated Ad vectors or nontreated Ad vectors, and supernatant was collected and measured for IFN-α by ELISA 24 h later. B, Cells that were transduced with 10,000 particles/cell were also stained with an Ab against CD86, and complete inactivation of the UV-treated vectors was verified by measuring GFP expression. C and D, C57BL/6, MyD88−/−, TLR3−/−, C3H/HeN, and C3H/HeJ BM-DCs were transduced with Ad vectors that express NP.S.EGFP or incubated with LPS or poly(I:C), and supernatant was collected and measured for IFN-α by ELISA 24 h later. ELISA data show mean ± SD for quadruplicate wells. Student’s t tests or ANOVA was performed, and p values < 0.05 are indicated by an asterisk (*). Data are representative of results from at least three independent experiments.

FIGURE 4. AdC68 and AdHu5 vectors induce IFN-α production through different pathways. 129Sv/Ev BM-DCs were preincubated with DMSO, 0.1 μm wortmannin, or 1 μm wortmannin for 1 h at 37°C and then transduced with Ad vectors that express NP.S.EGFP (10,000 particles vector/cell). A, Supernatants were collected and measured for IFN-α by ELISA 24 h later. Data show mean ± SD for quadruplicate wells. ANOVA was performed and p values < 0.05 are indicated by an asterisk (*). B, Cells were also stained with Abs against CD86. Data are representative of results from at least three independent experiments.
vectors (Fig. 6, A and B), which peaked at 10 days after injection. In 129Sv/Ev mice, we were able to detect strong transgene product-specific CD8+ T cell responses at 63 days after vaccination with either vector (4% of total CD8+ T cells). Because DCs in IFNAR−/− mice do not fully up-regulate costimulatory molecules after Ad injection, we expected that the generation of transgene product-specific CD8+ T cells would be impaired in these animals. Indeed, IFNAR−/− mice vaccinated with the AdHu5 vector initially generated SIINFEKL-specific CD8+ T cells at reduced frequencies during the primary response (Fig. 6A). However, at later time points, IFNAR−/− mice and 129Sv/Ev mice vaccinated with the AdHu5 vector achieved comparable levels of SIINFEKL-specific CD8+ T cells (Fig. 6A). After vaccination with the AdC68 vector, both IFNAR−/− mice and 129Sv/Ev mice mounted strong SIINFEKL-specific CD8+ T cell responses with identical kinetics (Fig. 6B). It is feasible that costimulatory molecules play a lesser role during the generation of CD8+ T cells directed against very high affinity epitopes, such as SIINFEKL. To address this concern, we measured NP-specific CD8+ T cell responses from splenocytes of the same animals described above and found that IFNAR−/− mice also develop NP-specific CD8+ T cells at frequencies comparable with those of 129Sv/Ev mice (Fig. 6C).

To verify that the CD8+ T cells generated in IFNAR−/− mice were not impaired, we tested SIINFEKL-specific CD8+ T cells for their TCR avidity and for additional functional properties. The TCR avidity of the SIINFEKL-specific CD8+ T cells isolated from
FIGURE 7. Transgene product-specific CD8\(^+\) T cells generated in Ad-vaccinated 129Sv/Ev and IFNAR\(^{-/-}\) mice have high TCR avidity and are functionally active. 129Sv/Ev and IFNAR\(^{-/-}\) mice were vaccinated i.m. with 1 \(\times\) 10\(^{10}\) particles of either AdHu5 or AdC68 vectors expressing IFNAR\(^{-/-}\) and 129Sv/Ev mice was the same after injection of either Ad vector (Fig. 7, A and B). SIINFEKL-specific CD8\(^+\) T cells isolated from IFNAR\(^{-/-}\) and 129Sv/Ev mice produced both IFN-\(\gamma\) and TNF-\(\alpha\) (Fig. 7C), indicating that these cells were not functionally impaired (54). Additionally, SIINFEKL-specific CD8\(^+\) T cells isolated from 129Sv/Ev and IFNAR\(^{-/-}\) mice translocated CD107a and CD107b to the cell surface upon peptide stimulation (Fig. 7D), indicating their potential for lytic activity (55). Thus, IFNAR\(^{-/-}\) mice apparently mount normal transgene product-specific CD8\(^+\) T cell responses without high levels of costimulatory molecules on DCs.

It is widely accepted that priming of naive CD8\(^+\) T cells requires presentation of Ag concomitantly with costimulatory molecules. We were able to detect a low percentage of CD11c\(^{high}\) DCs that expressed high levels of CD86 in the lymph nodes of unvaccinated 129Sv/Ev and IFNAR\(^{-/-}\) mice (Figs. 5 and 8A). We reasoned that these DCs, which exhibited a “preactivated” phenotype in naive IFNAR\(^{-/-}\) mice, could potentially be transduced with Ad vectors, leading to the priming of transgene product-specific CD8\(^+\) T cells. By using Ad vectors expressing only EGFP (which express higher overall levels of GFP compared with vectors expressing the NP.S.EGFP cassette), we measured transgene product expression in CD11c\(^{high}\) DCs removed from lymph nodes of vaccinated mice. Consistent with previous experiments, CD11c\(^{high}\) DCs isolated from the lymph nodes of Ad-vaccinated IFNAR\(^{-/-}\) mice expressed lower levels of CD86 compared with those from Ad-vaccinated 129Sv/Ev mice (Fig. 8, A and B). The dramatic increases in transgene expression that were found in cultured IFNAR\(^{-/-}\) BM-DCs were not seen in CD11c\(^{high}\) DCs isolated from Ad-vaccinated IFNAR\(^{-/-}\) mice. The effect of type I IFN on transgene expression may be less pronounced in vivo because secreted type I IFN is likely to bind to many other cell types that also express the type I IFNR, thus diluting the effect seen in cultured BM-DCs. Importantly, we could detect GFP-positive CD11c\(^{high}\) DCs that expressed high levels of CD86 in the lymph nodes of Ad-vaccinated IFNAR\(^{-/-}\) mice (Fig. 8B). Therefore, although global up-regulation of costimulatory molecules on DCs within Ad-vaccinated IFNAR\(^{-/-}\) mice is limited, there are small numbers of transduced DCs that do express high levels of costimulatory molecules. It is possible that these DCs were preactivated before vaccination and were transduced by the Ad vectors. Alternatively, this small subset of transduced DCs might have undergone maturation through a process that occurred independently of type I IFN signaling.

To verify that these DCs could directly prime naive CD8\(^+\) T cells, we vaccinated 129Sv/Ev or IFNAR\(^{-/-}\) mice with Ad vectors expressing NP.S.EGFP and isolated CD11c\(^{high}\) DCs from the lymph nodes and tested for their ability to stimulate transgenic OT1 CD8\(^+\) T cells in vitro. CD11c\(^{high}\) DCs isolated from 129Sv/Ev and IFNAR\(^{-/-}\) mice injected with either vector were able to stimulate the proliferation of OT1 CD8\(^+\) T cells (Fig. 8C).
After vaccination with the AdHu5 vector, there was no increase in transgene expression in CD11chigh DCs isolated from IFNAR−/− mice compared with those isolated from 129Sv/Ev mice (Fig. 8A), and there was no difference in the ability of these cells to induce proliferation of OT1 CD8+ T cells (Fig. 8C). After vaccination with the AdC68 vector, CD11chigh DCs isolated from IFNAR−/− mice induced the proliferation of OT1 CD8+ T cells more efficiently compared with CD11chigh DCs isolated from 129Sv/Ev mice. This could be related to transgene expression, because slightly higher levels of transgene product were found in CD11chigh DCs isolated from IFNAR−/− mice compared with 129Sv/Ev mice after vaccination with the AdC68 vector (Fig. 8A). Regardless, CD11chigh DCs isolated from IFNAR−/− mice injected with either Ad vector could induce the proliferation of Ag-specific CD8+ T cells, despite expressing low levels of costimulatory molecules.

**Discussion**

Ad vectors are attractive vaccine carriers because they induce strong transgene product-specific B and CD8+ T cell responses. This, in part, may relate to their ability to transduce DCs and cause these cells to undergo a maturation process. Recent data have indicated that type I IFN induced by some viruses drives DC maturation (33–35). In this study, we analyzed type I IFN involvement during Ad-mediated DC maturation. Using Ad vectors derived from a human serotype and a simian serotype, we show that Ad-transduced DCs produce type I IFN and that the type I IFNR is necessary for Ad-induced DC maturation.

In the United States, Africa, and Asia, as many as 45–80% of adult humans have high titers of neutralizing Abs directed against common human Ad serotypes (H. C. J. Ertl, unpublished data) (5), and these Abs can decrease the vaccine efficiency of vectors such
as AdHu5 (7). We developed alternative vectors based on simian Ad serotypes (such as AdC68) that do not circulate in the human population and that show no serological cross-reactivity with common serotypes of human Ads (5). In this study, we show that DCs transduced with AdC68 vectors produce more type I IFN and mature more efficiently compared with DCs transduced with AdHu5 vectors. Although we did not define the viral component of Ad that induces type I IFN production, it is likely a structural component because UV-inactivated AdC68 and AdHu5 vectors both induce type I IFN.

Several studies have suggested that capsid components of AdHu5 vectors trigger DC maturation; however, there have been conflicting reports on which capsid proteins are responsible. The hexon protein of AdHu5 acts as an adjuvant when coinfected with a model immunogen (15), whereas purified penton capsomere, full-length fiber protein, and the fiber knob are capable of inducing DC maturation in vitro (16). An AdHu5 vector with a fiber knob deletion (16) and an AdHu5 vector with a mutated penton sequence (17) both have reduced abilities to stimulate DCs. The different levels of type I IFN stimulated by AdC68 and AdHu5 vectors likely reflect structural differences in capsid components. AdC68 and AdHu5 vectors share 80 and 78% amino acid homology in the hexon and penton proteins, respectively, and 54% amino acid homology in the fiber protein.

We show that a phosphoinositide-3-OH kinase inhibitor blocks IFN-α production during transduction of DCs with an AdHu5 vector but not an AdC68 vector. DCs transduced with an AdHu5 vector produce TNF-α through a phosphoinositide-3-OH kinase-dependent mechanism, and this pathway is initiated by binding of the RGD motif of the penton base to integrins on DCs (17). Although the penton sequences of AdC68 and AdHu5 are fairly conserved, there is sequence divergence in regions flanking the RGD motif within the penton of each vector. This sequence variation may lead to structural differences in the RGD-containing domain of the penton of each vector, which could affect binding to integrins and subsequent activation of the phosphoinositide-3-OH kinase pathway. Further studies will determine the involvement of the penton and fiber proteins during Ad-induced type I IFN production.

In vivo, the major DC type transduced by both AdHu5 and AdC68 vectors are CD11chigh DCs (S. E. Hensley and H. C. J. Ertl, unpublished data). Here, we show that CD11chigh DCs isolated from wild-type mice vaccinated with an AdC68 vector express higher levels of costimulatory molecules but lower levels of transgene product compared with CD11chigh DCs isolated from wild-type mice vaccinated with an AdHu5 vector. Despite producing low levels of transgene product, AdC68 vectors induce strong transgene product-specific CD8+ T cell responses in wild-type mice. These responses are comparable with those from wild-type mice vaccinated with an AdHu5 vector, indicating that the magnitude of CD8+ T cell induction is not solely dependent on levels of transgene product in DCs during Ad vaccination. Initially, we hypothesized that AdC68 vectors induce strong transgene product-specific CD8+ T cell responses because they efficiently activate DCs. However, IFNAR−/− mice vaccinated with either AdHu5 or AdC68 vectors mount strong transgene product-specific CD8+ T cell responses despite expressing only marginal levels of costimulatory molecules on their DCs.

Based on our in vitro data, we expected that IFNAR−/− mice would not generate strong transgene product-specific CD8+ T cell responses after Ad vaccination. There are several explanations that could explain why these mice mount normal CD8+ T cell responses. First, we were able to detect a proportion of DCs in naive mice that expressed relatively high levels of costimulatory molecules, and it is possible that Ad vectors transduce these preactivated DCs. Indeed, whereas both AdHu5 and AdC68 vectors preferentially transduce immature DCs, they can also transduce mature DCs in vitro (11, 45). Second, we did find small increases in costimulatory molecule expression on DCs isolated from Ad-vaccinated IFNAR−/− mice, and it is possible these small increases are sufficient for the generation of transgene product-specific CD8+ T cell responses. In vivo, there may be additional cytokines or components other than type I IFN that can lead to partial DC maturation. Finally, Ad vectors may induce additional cytokines that directly promote the activation of CD8+ T cells in IFNAR−/− mice. Future studies will be designed to examine the roles of additional cytokines induced by Ad vectors during DC maturation and subsequent CD8+ T cell priming.

In summary, we show that two different Ad vectors stimulate type I IFN production through distinct mechanisms, and that both vectors induce DC maturation through pathways that are highly dependent on type I IFN signaling. Ad-induced type I IFN activates transduced DCs through an autocrine pathway and also activates bystander DCs. Although type I IFN is required for maximal up-regulation of costimulatory molecules after vaccination of Ad vectors in vivo, it is not required during the stimulation of transgene product-specific CD8+ T cell responses. This study further confirms the viral interactions with the innate immune system and highlights the sensitivity of CD8+ T cell priming during viral infections.

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Disclosures
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References


