TNF-α-dependent maturation of local dendritic cells is critical for activating the adaptive immune response to virus infection

Jose M. Trevejo*†, Michael W. Marino‡, Nicola Philpott*, Regis Josien§, Elizabeth C. Richards‡, Keith B. Elkon¶, and Erik Falck-Pedersen*‖

*Department of Microbiology and Immunology, WJ Hearst Foundation, Weill Medical College of Cornell University, New York, NY 10021; †The Cornell–Rockefeller–Sloan-Kettering Tri-Institutional MD-PhD Program, New York, NY 10021; ‡Ludwig Institute for Cancer Research, Memorial–Sloan Kettering Cancer Center, New York, NY 10021; §Service de Nephrologie-Immunologie Clinique, Centre Hospitalier Régional Universitaire de Nantes, Immeuble Jean Monnet, 44093 Nantes Cedex 01, France; and ¶Hospital for Special Surgery, New York, NY 10021

Communicated by Lloyd J. Old, Ludwig Institute for Cancer Research, New York, August, 10, 2001 (received for review March 5, 2001)

Tumor necrosis factor-α (TNF-α) is well recognized for its role in mediating innate immune responses. However, the mechanisms of TNF-α that influence the adaptive immune response to virus infections are not well understood. In this study, we have investigated the role of TNF-α in activating the cellular and humoral responses to systemic viral challenge with recombinant replication-defective adenovirus (rAd). Evaluation of T cell function in TNF-α-deficient (TNFKO) mice revealed impaired virus-specific proliferation of T cells derived from the draining lymph nodes of the liver. Analysis of dendritic cells (DC) isolated from local draining lymph nodes after systemic challenge showed that DC from TNFKO mice were relatively immature compared with those from strain-matched wild-type mice. *In vitro*, TNF-α was required to mature DC efficiently during virus-mediated stimulation. Adoptive transfer of primed, mature DC into TNFKO mice restored T cell responses and reconstituted anti-adenovirus antibody responses. Thus, TNF-α plays a significant role in the maturation of DC after adenovirus challenge both *in vitro* and *in vivo*, highlighting the importance of this innate cytokine in activating adaptive immunity to viral challenge.

Elucidating the innate mechanisms that influence adaptive immunity in viral infections is critical to understanding how an appropriate immune response is established (1). The immunogenic nature of replication-deficient adenoviruses (rAd) has made them a useful model for studying the host response to virus infection (1, 2). Immune responses to systemically administered rAd in mice include activation of both early and late phases of the immune system (3, 4). The early phase occurs in the first 2 days after infection and is characterized by early cytokine expression, including tumor necrosis factor-α (TNF-α) and IFN-γ, coupled with a polymorphonuclear infiltration of the liver (5, 6). During the late phase a potent antigen-specific $CD4^+$ and $CD8^+$ T cell response occurs (2). These innate and adaptive immune responses result in efficient clearance of rAd-transduced cells (2). Furthermore, induction of neutralizing antibodies prevents efficient readministration of rAd (1).

Critical to the initiation of adaptive immune responses to rAd and other viruses are dendritic cells (DC) (7, 8). DC are present in nonlymphoid organs such as the liver and skin in an immature state. During the course of an infection, DC take up viral particles, mature, and migrate to the local draining lymph node, where they efficiently activate both T and B cells (8). Maturation of DC involves substantial up-regulation of MHC class II and costimulatory molecules such as B7-1 and B7-2, thus making DC the most potent antigen-presenting cells in the immune repertoire (8).

A wide variety of viruses have evolved mechanisms to attenuate the host immune response (9, 10). Many of these viral strategies are directed toward abrogating the effects of TNF and the interferons (9), thus implicating these cytokines as critical to the antiviral immune response. Evidence for the importance of TNF in rAd infection has also been shown in studies in which *in vivo* expression of TNF-signaling inhibitors by rAd has attenuated inflammation and prolonged rAd-mediated transgene expression (11).

Generation of TNF-α-deficient (TNFKO) mice has facilitated investigation of the role for TNF in the immune response to rAd and other pathogens (12–15). Characterization of the immune response to rAd in TNFKO mice has shown cellular and humoral deficiencies relating to clearance of rAd-mediated transgene expression and generation of anti-Ad antibodies (14, 15).

To understand further the antiviral immune abnormalities of TNFKO mice, we conducted experiments to elucidate the mechanisms responsible for their compromised rAd response. Previous studies have shown that after i.v. rAd administration, the majority of virus localizes to the liver (3, 16) and is cleared by liver macrophages or Kupffer cells (5, 6). Consistent with these findings, we have demonstrated a T cell response to systemic rAd administration in draining lymph nodes of the liver that was compromised in TNFKO mice. Because DC play an important role in initiation of the immune response to rAd and other viruses (7), we hypothesized that the immune defect in TNFKO mice is directly related to impaired DC activation.

Materials and Methods

Mice and Viruses. TNFKO mice and their wild-type (WT) littermates were generated on a C57BL/6 $\times$ 129/Sv background and have been described elsewhere (13). C57BL/6, 129, and BALB/c mice were purchased from The Jackson Laboratory. Ad5CAT, Adβgal, and Ad5GFP are first-generation, $E1^-$ $E3^-$ replication-deficient adenoviruses carrying genes encoding chloramphenicol acetyltransferase (CAT), β-galactosidase, and green fluorescent protein, respectively, and have been described (14).

T Cell Proliferation Lymphocytes were taken from the portal lymph nodes that have been described by Tilney (17) and shown to drain the liver. The methods used to measure viral specific T cell responses have been described (15). Single-cell suspensions of lymphocytes were plated out in triplicate in a 96-well U-bottom plate at 5×10^5 cells per well in RPMI medium 1640 + 5% FBS (R5). Restimulated cells were treated with 2,000

Abbreviations: BMDC, bone marrow-derived dendritic cells; CAT, chloramphenicol acetyltransferase; DC, dendritic cells; GFP, green fluorescent protein; MLR, mixed lymphocyte reaction; PE, phycoerythrin; rAd, recombinant replication-defective adenovirus; TNF, tumor necrosis factor; TNFKO, TNF-α-deficient (knockout); WT, wild type.

‖To whom reprint requests should be addressed. E-mail: efalckp@med.cornell.edu.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

particles per cell of AdGFP or AdCAT. Cells were incubated for 2 days with the last 8 h in the presence of 1 μCi/200 μl [^{3}H]thymidine (1 μCi = 37 kBq), and incorporation was measured. BrdUrd measurement of proliferation was performed by plating 2.5×10^5 cells in a 24-well plate with or without 2,000 particles of AdCAT per ml. Cells were incubated for 2 days in the presence of a final concentration of 10 μM BrdUrd added every 12 h. Cells were harvested, stained for CD8 or CD4 (PharMingen), permeabilized, and fixed as described (18). Cells were then counterstained with FITC-conjugated anti-BrdUrd (Becton Dickinson), and the percent of BrdUrd incorporation was determined by flow cytometry.

Bone Marrow DC. Bone marrow-derived DC (BMDC) were isolated from WT and knockout (KO) mice by using the method of Inaba *et al.* (19). Bone marrow from mice was isolated and the red blood cells were lysed. Bone marrow cells were then placed in 24-well plates at 1×10^6 per ml, 1 ml per well, in R5 plus granulocyte–macrophage colony-stimulating factor. Cells were gently washed on days 2 and 4 to remove granulocytes and harvested on day 6. Day 6 cells were replated at 1×10^6 per ml in 10-cm plates.

Mixed Lymphocyte Reaction (MLR). Allostimulatory capacity was measured as described (20). Allogeneic T cells from BALB/c mice (H-2K^D) were purified from the spleen and lymph nodes by using positive magnetic selection of cells stained with Thy-1.3 bead-conjugated antibody (Miltenyi Biotec, Auburn, CA). T cells were plated in U-bottom plates at 1×10^5 per well. BMDC were added to T cells at 5-fold dilutions in triplicate starting with 5×10^4 DC per well. The cells were incubated for 3 days with the last 8 h in the presence of [^{3}H]thymidine, and incorporation was measured.

DC Addback. Day 6 WT or TNFKO-derived BMDC were infected with either AdCAT or AdGFP at 8,000 particles per cell for 20 min in serum-free RPMI medium 1640. Cells were resuspended in R5 + granulocyte-macrophage colony-stimulating factor and incubated for 48 h. Day 8 infected DC were washed three times with sterile PBS and resuspended at $2–5 \times 10^5$ cells in 100 μl of PBS and injected intravenously. To measure T cell proliferation after transfer, mice were challenged with rAd 2 days after transfer and assayed as described above on day 10 after infection. For CAT expression and antibody induction, mice were challenged with rAd 7 days after transfer and assayed for antibody as described above on day 21 after infection. CAT assays were also performed on day 21 as described (14).

Analysis of DC Surface Phenotype. DC were stained on ice with rat monoclonal antibodies for 30 min in PBS with 1% FBS. Cells were fixed and analyzed by flow cytometry. The following antibodies were used: phycoerythrin (PE)-anti CD86, PE-anti-CD8, PE-anti-CD4, PE-and FITC-anti-CD11c, and biotinylated anti-H-2K^b (PharMingen).

Results

Impaired T and B Cell Responses in TNFKO Mice. On the basis of the compromised cellular and humoral immune response to rAd in TNFKO mice (14, 15), we hypothesized that the absence of TNF may result in suboptimal activation of T cells. To determine whether TNFKO mice are compromised in their T cell response to rAd, we measured proliferation of the portal lymph node T cell populations [portal lymph nodes have been shown to drain the liver (17)] 10 days after systemic rAd administration. Lymphocytes isolated from portal lymph nodes were restimulated with rAd, and specific T cell proliferation was measured by [^{3}H]thymidine incorporation (Fig. 1*A*). WT mice infected with rAd showed a strong adenovirus-specific proliferative response.

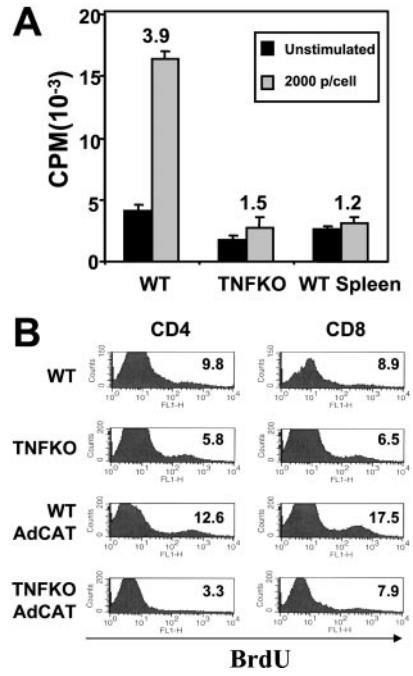
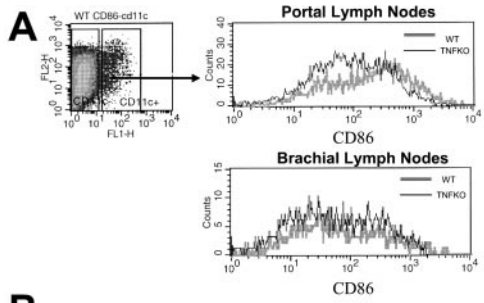


Fig. 1. Compromised T cell activation in TNFKO mice. (*A*) TNFKO and WT mice ($n = 3$) were challenged with AdGFP as indicated in *Materials and Methods*. Ten days later portal lymph nodes that drain the liver were collected and pooled, and single-cell suspensions were restimulated *in vitro* for 3 days with or without 2,000 particles per cell (p/cell) of AdGFP. Proliferating T cells were labeled with [^{3}H]thymidine. The results are expressed as the mean of triplicates and are representative of two experiments. The number above each bar is the ratio of stimulated to unstimulated proliferation. (*B*) Mice were challenged as in *A*, except AdCAT was used and cells were restimulated with 2,000 particles per cell of AdCAT. BrdUrd (BrdU) was added to a final concentration of 10 μM during culture. The cells were harvested after 48 h and stained with CD8 or CD4 and FITC-anti-BrdUrd antibody. The numbers in each histogram represent percent BrdUrd-positive cells as measured by flow cytometry.

Lymphocytes isolated from TNFKO lymph nodes were nonresponsive to rAd stimulation in this assay. Splenocytes failed to show significant rAd-specific responses in either WT or TNFKO mice (Fig. 1*A*). Further analysis of the T cell proliferation in response to rAd stimulation (Fig. 1*B*) demonstrated a dominant CD8$^+$ T cell response and an induction of CD4$^+$ T cells in WT animals. Neither population was measurably activated in the TNFKO mouse (Fig. 1*B*). Analysis of antibody responses also showed a failure to induce anti-adenovirus IgG antibodies in TNFKO mice even after 8 weeks (data not shown). Therefore, systemic administration of rAd induces a local T cell activation in WT animals that is absent or compromised in TNFKO mice. Furthermore, TNFKO mice have impaired humoral responses to rAd.

TNFKO Mice Fail to Fully Mature DC in Response to Adenovirus. Compromised local T cell activation after systemic administration of rAd in TNFKO animals suggested a previously uncharacterized defect in the cellular immune activation cascade. Bone-marrow-derived DC are one of the primary antigen-presenting cells responsible for cytotoxic T cell activation to viral antigens (21). If the observed TNFKO T cell defect was caused by compromised stimulation by DC, then we would predict that the DC population from the local lymph node would be decreased or relatively immature after rAd challenge. To determine whether TNFKO mice have such a deficiency, portal lymph nodes were isolated 48 h after challenge and isolated cells were double stained with a dendritic cell-specific marker CD11c and



B

Hepatic DLN	CD11c (%)	CD86 Mean Flourescence	CD86 Peak Channel
WT	4.31	345	296
TNFKO	5.89	154	55
Brachial DLN			
WT	1.32	164	36
TNFKO	2.05	129	17

Fig. 2. Compromised maturation of local lymph node DC in TNFKO mice. WT and TNFKO mice ($n = 3$) were challenged with AdGFP. After 48 h, portal and brachial lymph nodes were collected and pooled, and single-cell suspensions were isolated. Cells were double stained for CDIlc and CD86 and analyzed by flow cytometry (*A*). Numerical flow data are also shown (*B*). DNL, draining lymph nodes. The results are representative of two experiments.

the costimulatory marker CD86 (B7-2). The percentages of DC ($CD11c^+$) present in WT and TNFKO unchallenged animals were similar at 1.9% and 1.7%, respectively ($n = 3$) (data not shown). The percentages of WT and TNFKO $CD11c^+$ cells present in the portal lymph nodes after rAd stimulation increased to 4.3% in WT and 5.8% in TNFKO mice (Fig. 2*B*). However, when CD86 expression was used as a marker for the maturation stage of the respective DC populations, only the challenged WT mice demonstrated a significant shift toward a more mature DC population (Fig. 2). Brachial lymph nodes that do not drain the liver were isolated from the same mice that were challenged above. Analysis of these nodes did not show a change in either WT or TNFKO mice, consistent with local activation in the liver after systemic rAd challenge (Fig. 2). Therefore, TNFKO mice did not seem to have a DC migration defect, but did have a compromised maturation of local DC in response to systemic rAd infection.

Compromised Function of TNFKO BMDC. Because the observations above suggested defective maturation of DC in TNFKO mice, the role of TNF in DC maturation was further examined by using myeloid DC. Bone marrow precursors were isolated from WT and TNFKO animals and cultured in the presence of granulocyte-macrophage colony-stimulating factor under conditions that induce DC differentiation and progressive maturation (19). This *ex vivo* method of DC generation usually results in maturation of DC by day 8 of the culture (19). Flow cytometric analysis of day 8 BMDC revealed that TNFKO-derived DC were significantly less mature than WT DC as determined by expression of the markers class II, CD86 (B7-2), and CD40 (Fig. 3*A*). High expression levels of these markers are indicative of DC maturation. Using the MLR as a measure of DC maturity and function, we determined that TNFKO-derived DC also displayed decreased T cell-stimulatory capacity compared with WT-derived DC in an allogeneic MLR (Fig. 3*B*). We sorted TNFKO DC for a $CD86^+$ subset to create a purified population of mature TNFKO DC. This mature subset of TNFKO DC demonstrated T cell stimulation potency comparable with WT DC (Fig. 3*C*), illustrating that lack of sufficient numbers of mature DC, rather than inherent abnormalities, was responsible for the compromised MLR activity of DC from TNFKO mice.

Because BMDC cultures from TNFKO mice lack expression of TNF, we sought to determine whether addition of TNF would reconstitute DC maturation and MLR-stimulatory capacity. TNFKO- and WT-derived BMDC were cultured in the presence or absence of 500 pg/ml recombinant mouse TNF-α. DC were washed to remove any residual TNF before addition to the MLR. Analysis of surface markers showed that culture of the BMDC with TNF resulted in increased maturation of the TNFKO-

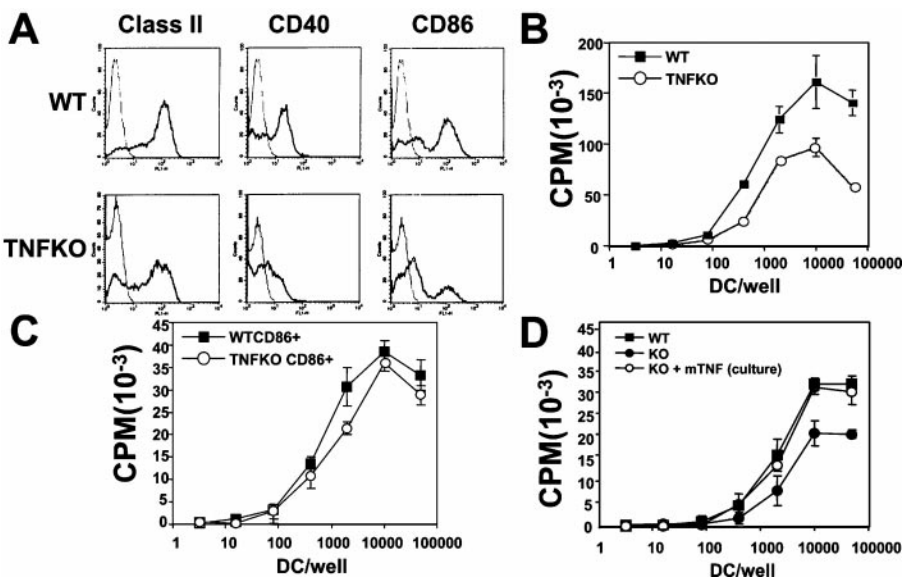


Fig. 3. Decreased maturity and allostimulatory capacity of TNFKO-derived DC. (*A*) Day 8 WT and TNFKO BMDC were stained with surface markers as indicated and analyzed by flow cytometry. The results are representative of three experiments. (*B*) Day 8 WT and TNFKO BMDC were coincubated with T cells for 3 days, and [^{3}H]thymidine incorporation was measured. The results are expressed as the mean of triplicates and are representative of three experiments. (*C*) Day 8 BMDC were stained for CD86 and sorted for the $CD86^+$ population. $CD86^+$-sorted WT and TFNKO BMDC were assayed as in *B*. The results are expressed as the mean of triplicates and are representative of two experiments. (*D*) TNFKO BMDC cultures were incubated for 8 days with or without mouse TNF-α (mTNF; 500 pg/ml). WT BMDC were incubated without additional TNF. DC allostimulatory capacity was assayed as in *B*. The results are the mean of triplicates and are representative of two experiments.

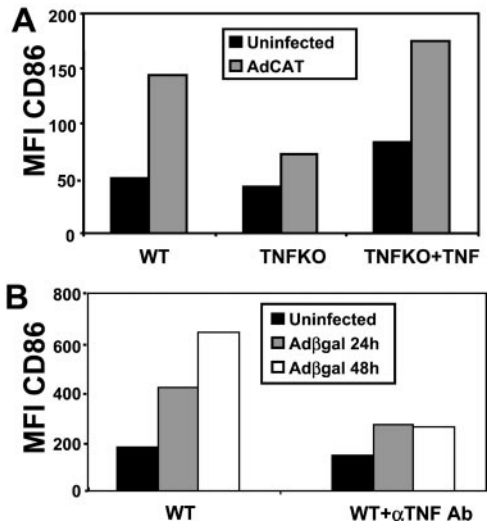


Fig. 4. TNF potentiates adenovirus-mediated maturation of DC. (*A*) Day 5 immature WT and TNFKO BMDC were incubated for 24 h with or without rAd at 5,000 particles per cell. For TNFKO DC treated with TNF, recombinant murine TNF-α (5 ng/ml) was added. (*B*) Same as *A*, except WT DC were treated with anti-TNF-α antibody (5 μg/ml). DC were stained with anti-CD86 PE. Cells were analyzed by flow cytometry and the mean fluorescence intensity (MFI) of CD86 surface expression was determined. The results are representative of three experiments.

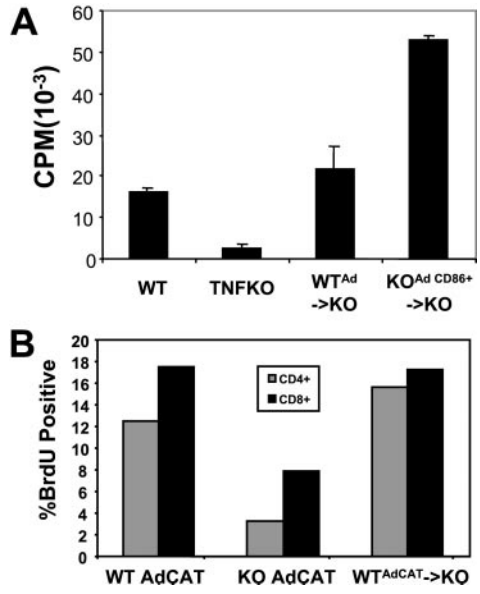


Fig. 5. Adoptive transfer of mature DC corrects the T cell response to adenovirus administration. (*A*) Day 8 infected BMDC were washed and adoptively transferred into naive TNFKO mice (5×10^5 per mouse). Two days after transfer naive TNFKO, WT, TNFKO, WT BMDC, and TNFKO + CD86$^+$ TNFKO BMDC mice were challenged with 1×10^{10} particles of AdGFP. On day 10 portal lymph nodes were collected and pooled, and single-cell suspensions were restimulated with AdGFP. Proliferating T cells were labeled with [^{3}H]thymidine for the last 8 h. The results are the mean of triplicate assays and are representative of two experiments. (*B*) BMDC were infected as in *A*, except AdCAT was used. Mice were challenged with 1×10^{10} particles of AdCAT, and single-cell suspensions were restimulated with AdCAT. During the culture, 1 μM BrdUrd was added. The cells were harvested after 48 h and stained with CD8 or CD4 and FITC-anti-BrdUrd antibody. Percent BrdUrd-positive cells was measured by flow cytometry.

derived DC to WT levels (data not shown). The BMDC that received TNF during culture were then used in an MLR assay and shown to stimulate with equal efficiency to WT DC (Fig. 3*D*). However, addition of TNF directly to the MLR did not reconstitute the T cell allostimulatory capacity of TNFKO DC to WT levels (data not shown). Thus, addition of TNF to the TNFKO BMDC culture resulted in increased maturation and reconstitution of the TNFKO DC-mediated stimulation during an MLR.

Previous studies have shown that rAd infection of DC stimulates maturation, cytokine expression, and NF-κB induction (22). Our studies have shown deficiencies of DC maturation in TNFKO mice, implying that rAd infection coupled with TNF expression may act synergistically to induce DC maturation. To examine this question, maturation of day 5 WT and TNFKO BMDC was evaluated by CD86 expression 24 or 48 h after rAd infection (Fig. 4). Day 5 BMDC are immature and therefore representative of the immature DC that are resident in tissues and organs. Infection of DC with rAd induced a greater maturation of WT DC than TNFKO DC (Fig. 4*A*). The addition of recombinant TNF to TNFKO BMDC resulted in marked enhancement of maturation of TNFKO DC. Furthermore, blockage of TNF with anti-TNF antibody during infection prevented efficient maturation of WT DC (Fig. 4*B*). These experiments illustrate that rAd promotes DC maturation and in the presence of TNF, this maturation is significantly more efficient. Because DC have been shown to express TNF after rAd infection (22), autocrine stimulation by TNF could be responsible for the increased maturation of WT DC.

Adoptive Transfer of rAd-Primed DC Corrects Immune Deficiencies of TNFKO Mice. The data presented above suggest that defective immune responses to rAd in TNFKO mice are caused by compromised DC maturation. Adoptively transferred DC have been shown to home to the liver and then migrate via the liver lymphatics to the draining hepatic lymph nodes, where they efficiently activate the immune system (23). Therefore, we predicted that the immune defect in TNFKO mice could be complemented if BMDC, matured and primed with rAd, were adoptively transferred into TNFKO mice. As a negative control, the same BMDC matured in the absence of rAd would not be expected to complement the TNFKO defect because of the lack of presentation of rAd antigens. After adoptive transfer of 2×10^5 rAd-primed WT DC, TNFKO mice were challenged and rAd-specific T cell proliferation was determined (Fig. 5*A*). Cellular activation was measured and T cell proliferation was shown to be restored to WT levels. Addback of DC reconstituted both the CD4$^+$ and CD8$^+$ proliferation as measured by BrdUrd staining of TNFKO mice receiving rAd-primed DC (Fig. 5*B*). The above *in vitro* data suggest that the maturity status of the DC was relevant to efficient T cell activation. Therefore, it was also important to confirm that mature TNFKO DC were able to reconstitute immunity in TNFKO mice. To address this issue, TNFKO BMDC were infected at day 5, and the CD86$^+$ population was sorted to isolate the mature subset of rAd-primed, mature TNFKO DC (Fig. 5*A*). Adoptive transfer of these DC reconstituted T cell activation in TNFKO mice to levels even greater than unsorted, primed WT DC, demonstrating that mature TNFKO DC alone are sufficient to activate rAd-specific T cell responses in TNFKO mice (Fig. 5*A*). Histology of livers from TNFKO mice receiving DC showed increased infiltration compared with TNFKO mice that were challenged, but did not receive primed DC (data not shown).

Complementation of the cellular defect in TNFKO mice, as measured by clearance of cells expressing the CAT transgene after AdCAT infection, indicated enhanced but incomplete clearance of CAT-expressing hepatocytes (Fig. 6*A*). Adoptive transfer of DC was also able to reconstitute the humoral

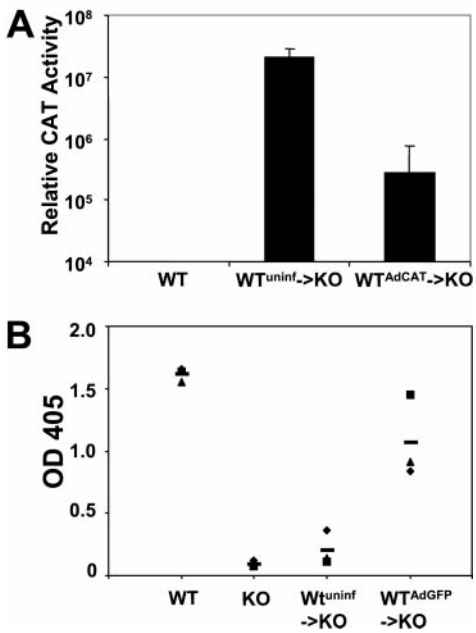


Fig. 6. Adoptive transfer of mature DC reconstitutes clearance of AdCAT expression and corrects the antibody response to adenovirus in TNFKO mice. (*A*) Day 6 BMDC obtained from WT mice were infected with AdCAT (8,000 particles per cell) for 48 h in the presence of granulocyte-macrophage colony-stimulating factor. Day 8 infected BMDC were washed and adoptively transferred into naive TNFKO mice (2×10^5 per mouse). After 7 days the mice were challenged with 1×10^{10} particles of AdCAT, and CAT expression was assayed on day 21 after the challenge. Bars are averages of three mice. (*B*) Mice were treated as in *A*, except AdGFP was used. After 7 days the mice were challenged with 1×10^{10} particles of AdGFP, and their serum was assayed for anti-adenovirus antibodies on day 21 after the challenge. Indirect ELISA of mouse IgG was performed with whole adenovirus particles as the target antigen. Serum was diluted 1:1000. Horizontal bars are average of three mice. The results are representative of two experiments.

response in TNFKO mice when antibody responses were measured (Fig. 6*B*). Anti-Ad titers were comparable with those found in WT animals when challenged with rAd (Fig. 6*B*). In comparison, animals that received naive WT DC generated baseline anti-Ad titers. Purified WT B cells from noninfected mice also failed to complement the TNFKO antibody deficiency (data not shown), thus confirming that reintroduction of WT TNF-expressing cells alone is not sufficient to correct TNFKO mice. Therefore, adoptive transfer of primed, mature DC was sufficient to correct both the humoral and cellular responses to systemic rAd administration in TNFKO mice.

Discussion

This study uses rAd infection of mice as a model to examine the mechanisms of TNF-α-mediated viral immunity in response to systemic infection. Because these viruses are replication-defective, immune activation primarily depends on the structure of the virion along with the process of initial viral entry and transgene expression (24). Therefore, this model of virus infection is particularly relevant to the initial events defining the immune response to a viral infection. Systemic administration of rAd results in predominant localization of virions to the liver (3, 16). In addition to hepatocytes, immune cells resident in the liver such as macrophages and dendritic cells can take up viral particles (6). Uptake by these cells results in innate immune activation and subsequent viral-specific T cell activation by mature DC presenting viral antigens (5, 7). Maturation and proper education of DC depends on microbial stimulation, which occurs during virus uptake and in many cases depends on recognition of patterns inherent to the pathogen (25–27). Furthermore, other signals such as cytokines synergize with microbial stimulation to facilitate DC maturation (28, 29).

The host response to intracellular pathogens such as adenovirus relies on activation of the Th1 pathway, which seems to be selectively deficient in TNFKO mice (12–14). In liver infection, T cell activation occurs in the portal lymph nodes, which along with parathymic lymph nodes have been shown to be the primary nodes draining the liver in mice and rats (17). A potent $CD4^+$ and $CD8^+$ T cell response has been shown to be relevant to clearance of rAd expression (2, 24). Our studies determined that both the $CD4^+$ and $CD8^+$ portal lymph node T cells from TNFKO mice were nonresponsive to rAd infection (Fig. 1 *A* and *B*). In contrast, lymphocytes from WT mice that were challenged systemically displayed significant adenovirus-specific proliferation. Previous reports studying TNFKO mice have not explicitly identified T cell defects (12–14), perhaps because the lymphocytes from the spleen rather than the local draining lymph nodes were analyzed. In our model, splenic derived cells from WT mice did not display significant T cell proliferation differences relative to the draining lymph nodes, highlighting the importance of local immune activation (Fig. 1*A*).

Deficiencies of both the humoral and cellular immune response led us to hypothesize a common mechanism that was upstream of T cell activation. Because DC mediate efficient viral-specific T cell stimulation, we hypothesized that these mice had deficient DC function. To determine the role of TNF in virus-induced DC maturation, we analyzed TNFKO mice for DC deficiencies in response to rAd challenge. Normal percentages of DC were detected in the spleen and lymph node of TNFKO mice (data not shown), suggesting that there is no abnormality in differentiation of DC from bone marrow precursors. In addition, after rAd administration, similar increases in $CD11c^+$ DC were identified in both TNFKO and WT lymph nodes draining the liver. However, when DC were analyzed for expression levels of the maturation marker CD86 (B7-2), those from TNFKO mice expressed significantly lower levels than their WT counterparts. Compromised maturation of hepatic DC in TNFKO mice is proposed to account directly for the *in vivo* deficiency of liver draining lymph node T cell activation. Because both $CD4^+$ and $CD8^+$ T cells were nonresponsive to rAd infection in TNFKO mice, deficiency of DC maturation provides a clear explanation for the attenuation of cellular and humoral immune responses. Previous studies have shown a role for TNF-mediated Langerhans cell migration in the skin (29–32). TNF has also been shown to increase the migration of DC from the skin, kidney, and heart (33) and increase maturation and motility of DC *in vitro* (34). TNF-dependent maturation of hepatic DC may explain previous observations of attenuated rAd responses in TNFKO mice (14), as well as WT mice receiving rAd expressing a soluble inhibitor of TNF (15).

To confirm our hypothesis of compromised TNFKO DC maturation *in vivo*, we demonstrated that DC were able to reconstitute both cellular and humoral immune responses by adoptive transfer of rAd-primed BMDC. Previous studies have determined that adoptively transferred, mature DC will traffic to the draining hepatic lymph nodes in the same manner as endogenous DC (23), thus supporting our model of DC reconstitution. After adoptive transfer, the TNFKO mice that received primed, mature WT or TNFKO DC were able to mount normal antibody and T cell responses and clear rAd-mediated transgene expression (Figs. 5 and 6). These results illustrate that lack of activation caused by decreased DC maturation is one of the primary reasons for the immune deficiencies observed in TNFKO mice. These results give further *in vivo* evidence for the importance of TNF in early DC maturation in addition to its well-defined role in apoptosis and inflammation. It is unclear whether signaling of these functions of TNF occurs through

TNFR1 or TNFR2, although both receptors have been important in mediating antiviral effects of TNF (35). Furthermore, Minter *et al.* (36) have shown a requirement for both receptors in response to rAd challenge, suggesting that both may be important for efficient DC activation.

Our model for the role of TNF in the anti-adenoviral response emphasizes that both microbial stimulation and local TNF expression contribute to efficient maturation and migration of DC. A systemic challenge of adenovirus leads to uptake by immature DC and Kupffer cells in the liver. The role of Kupffer cells in rAd-mediated TNF expression has been highlighted in previous studies showing Kupffer cell-dependent expression of TNF within the first hour of rAd infection (5). On the basis of our studies, exposure to rAd in the presence of TNF is critical for efficient DC maturation and migration. Activation of macrophages and DC by rAd particles has been shown in both mouse and primate models, consistent with the notion of rAd-mediated stimulation of professional antigen-presenting cell (37, 38). Because of the observed increase in the percentage of the portal lymph node DC population after rAd infection in both WT and TNFKO mice, there may be a lesser role for TNF in mediating migration of DC compared with maturation in our system. DC that have both taken up rAd and receive an autocrine or paracrine signal from TNF undergo efficient maturation and migrate to the draining lymph node, where they initiate potent T and B cell responses. This model does not exclude the role of other cytokines and molecules in the maturation of DC. In fact, IL-1 and CD40 have both been shown to stimulate DC maturation and may work together with TNF for efficient immune activation (28, 39). Furthermore, in this model, the $CD11c^+$ DC or myeloid lineage DC may also elaborate TNF after virus uptake (22), thus amplifying TNF-mediated maturation locally and in the draining lymph node.

It is now becoming increasingly apparent that initial events that result in professional antigen-presenting cell activation have a strong influence on the shaping of our adaptive immune responses to viruses and other pathogens (27). The activation of DC and macrophages seems to be mediated in part by recognition of inherent viral patterns by the Toll/IL-1 receptor family leading to expression of inflammatory cytokines such as TNF (26, 40, 41). Our data underscore the role of TNF as a bridge between initiation of the inflammatory cascade and generation of the antigen-specific response. Further investigation of this complex interplay between the innate and adaptive immune responses will have profound implications in viral immunology and vaccine development.

We thank Drs. Brian Wong and Yufeng Peng for helpful discussions and reagents and Dr. Lloyd Old for critical review of the manuscript and generous supply of animals. This work was supported in part by National Institutes of Health Grant HL51746 (to E.F.P. and K.B.E.). J.M.T. was supported by The W.M. Keck Foundation Medical Scientist Fellowship, The Minority Predoctoral Fellowship Program GM18268, and National Institutes of Health Medical Scientist Training Program Grant GM07739.

1. Yang, Y., Li, Q., Ertl, H. C. & Wilson, J. M. (1995) *J. Virol.* **69,** 2004–2015.
2. Yang, Y., Ertl, H. C. & Wilson, J. M. (1994) *Immunity* **1,** 433–442.
3. Worgall, S., Wolff, G., Falck-Pedersen, E. & Crystal, R. G. (1997) *Hum. Gene Ther.* **8,** 37–44.
4. Benihoud, K., Saggio, I., Opolon, P., Salone, B., Amiot, F., Connault, E., Chianale, C., Dautry, F., Yeh, P. & Perricaudet, M. (1998) *J. Virol.* **72,** 9514–9525.
5. Lieber, A., He, C. Y., Meuse, L., Schowalter, D., Kirillova, I., Winther, B. & Kay, M. A. (1997) *J. Virol.* **71,** 8798–8807.
6. Wolff, G., Worgall, S., van Rooijen, N., Song, W. R., Harvey, B. G. & Crystal, R. G. (1997) *J. Virol.* **71,** 624–629.
7. Jooss, K., Yang, Y., Fisher, K. J. & Wilson, J. M. (1998) *J. Virol.* **72,** 4212–4223.
8. Banchereau, J. & Steinman, R. M. (1998) *Nature (London)* **392,** 245–252.
9. Krajcsi, P. & Wold, W. S. (1998) *Semin. Cell Dev. Biol.* **9,** 351–358.
10. Mahr, J. A. & Gooding, L. R. (1999) *Immunol. Rev.* **168,** 121–130.
11. Ilan, Y., Droguett, G., Chowdhury, N. R., Li, Y., Sengupta, K., Thummala, N. R., Davidson, A., Chowdhury, J. R. & Horwitz, M. S. (1997) *Proc. Natl. Acad. Sci. USA* **94,** 2587–2592.
12. Pasparakis, M., Alexopoulou, L., Episkopou, V. & Kollias, G. (1996) *J. Exp. Med.* **184,** 1397–1411.
13. Marino, M. W., Dunn, A., Grail, D., Inglese, M., Noguchi, Y., Richards, E., Jungbluth, A., Wada, H., Moore, M., Williamson, B., *et al.* (1997) *Proc. Natl. Acad. Sci. USA* **94,** 8093–8098.
14. Elkon, K. B., Liu, C. C., Gall, J. G., Trevejo, J., Marino, M. W., Abrahamsen, K. A., Song, X., Zhou, J. L., Old, L. J., Crystal, R. G. & Falck-Pedersen, E. (1997) *Proc. Natl. Acad. Sci. USA* **94,** 9814–9819.
15. Peng, Y., Trevejo, J., Zhou, J., Marino, M. W., Crystal, R. G., Falck-Pedersen, E. & Elkon, K. B. (1999) *J. Virol.* **73,** 5098–5109.
16. Vrancken Peeters, M. J., Perkins, A. L. & Kay, M. A. (1996) *BioTechniques* **20,** 278–285.
17. Tilney, N. L. (1971) *J. Anat.* **109,** 369–383.
18. Tough, D. F. & Sprent, J. (1998) *Vet. Immunol. Immunopathol.* **63,** 123–129.
19. Inaba, K., Inaba, M., Romani, N., Aya, H., Deguchi, M., Ikehara, S., Muramatsu, S. & Steinman, R. M. (1992) *J. Exp. Med.* **176,** 1693–1702.
20. Wong, B. R., Josien, R., Lee, S. Y., Sauter, B., Li, H. L., Steinman, R. M. & Choi, Y. (1997) *J. Exp. Med.* **186,** 2075–2080.
21. Sigal, L. J. & Rock, K. L. (2000) *J. Exp. Med.* **192,** 1143–1150.
22. Morelli, A. E., Larregina, A. T., Ganster, R. W., Zahorchak, A. F., Plowey, J. M., Takayama, T., Logar, A. J., Robbins, P. D., Falo, L. D. & Thomson, A. W. (2000) *J. Virol.* **74,** 9617–9628.
23. Kudo, S., Matsuno, K., Ezaki, T. & Ogawa, M. (1997) *J. Exp. Med.* **185,** 777–784.
24. Yang, Y., Nunes, F. A., Berencsi, K., Furth, E. E., Gonczol, E. & Wilson, J. M. (1994) *Proc. Natl. Acad. Sci. USA* **91,** 4407–4411.
25. Bachmann, M. F., Zinkernagel, R. M. & Oxenius, A. (1998) *J. Immunol.* **161,** 5791–5794.
26. Janeway, C., Jr., & Medzhitov, R. (2000) *Proc. Natl. Acad. Sci. USA* **97,** 10682–10683.
27. Brown, P. (2001) *Nature (London)* **410,** 1018–1020.
28. Jonuleit, H., Knop, J. & Enk, A. H. (1996) *Arch. Dermatol. Res.* **289,** 1–8.
29. Wang, B., Amerio, P. & Sauder, D. N. (1999) *J. Leukoc. Biol.* **66,** 33–39.
30. Kimber, I. & Cumberbatch, M. (1992) *J. Invest. Dermatol.* **99,** 48S–50S.
31. Cumberbatch, M. & Kimber, I. (1995) *Immunology* **84,** 31–35.
32. Wang, B., Fujisawa, H., Zhuang, L., Kondo, S., Shivji, G. M., Kim, C. S., Mak, T. W. & Sauder, D. N. (1997) *J. Immunol.* **159,** 6148–6155.
33. Roake, J. A., Rao, A. S., Morris, P. J., Larsen, C. P., Hankins, D. F. & Austyn, J. M. (1995) *J. Exp. Med.* **181,** 2237–2247.
34. Winzler, C., Rovere, P., Rescigno, M., Granucci, F., Penna, G., Adorini, L., Zimmermann, V. S., Davoust, J. & Ricciardi-Castagnoli, P. (1997) *J. Exp. Med.* **185,** 317–328.
35. Ruby, J., Bluethmann, H. & Peschon, J. J. (1997) *J. Exp. Med.* **186,** 1591–1596.
36. Minter, R. M., Rectenwald, J. E., Fukuzuka, K., Tannahill, C. L., La Face, D., Tsai, V., Ahmed, I., Hutchins, E., Moyer, R., Copeland, E. M., 3rd & Moldawer, L. L. (2000) *J. Immunol.* 164, 443–451.
37. Zhang, Y., Chirmule, N., Gao, G., Qian, R., Croyle, M., Joshi, B., Tazelaar, J. & Wilson, J. M. (2001) *Mol. Ther.* **3,** 697–707.
38. Schnell, M. A., Zhang, Y., Tazelaar, J., Gao, G., Yu, Q. C., Qian, R., Chen, S. J., Varnavski, A. N., LeClair, C., Raper, S. E. & Wilson, J. M. (2001) *Mol. Ther* **3,** 708–722.
39. Caux, C., Massacrier, C., Vanbervliet, B., Dubois, B., Van Kooten, C., Durand, I. & Banchereau, J. (1994) *J. Exp. Med.* **180,** 1263–1272.
40. Kurt-Jones, E. A., Popova, L., Kwinn, L., Haynes, L. M., Jones, L. P., Tripp, R. A., Walsh, E. E., Freeman, M. W., Golenbock, D. T., Anderson, L. J. & Finberg, R. W. (2000) *Nat. Immunol.* **1,** 398–401.
41. Bowie, A., Kiss-Toth, E., Symons, J. A., Smith, G. L., Dower, S. K. & O'Neill, L. A. (2000) *Proc. Natl. Acad. Sci. USA* **97,** 10162–10167. (First Published August 1, 2000; 10.1073/pnas.160027697)