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Interference with Dendritic Cell Populations Limits Early Antigen Presentation in Chronic γ-Herpesvirus-68 Infection

Adele M. Mount,* Frederick Masson,* Fiona Kupresanin,* Christopher M. Smith,† Janet S. May,‡ Nico van Rooijen,§ Philip G. Stevenson,‡ and Gabrielle T. Belz*

A critical factor influencing the ability of the host to mount a robust immune response against a virus depends on the rapid recruitment of dendritic cells (DCs) presenting Ags. From the outset, this step sets the tempo for subsequent activation of virus-specific T cells. Despite this, how induction of the immune response might be modified by pathogens with the capacity to establish persistence is unclear. In this study, we have characterized the in vivo influence of murine γ-herpesvirus K3-mediated interference with MHC class I in DCs that drive the initial adaptive immune response. We observed that γ-herpesvirus could interfere with the very earliest phase of Ag presentation through K3 by directly targeting migratory and lymph node-resident DCs. These results show that a pathogen with the capacity to interfere with early Ag presentation can establish suboptimal conditions for rapid induction of the adaptive immune response and thus favor establishment of viral persistence. The Journal of Immunology, 2010, 185: 3669–3676.

Dendritic cells (DCs) are involved in a wide range of immune responses ranging from resistance to infection to maintenance of self-tolerance. Importantly, different DC subsets appear to have specialized roles in immune responses, such as the secretion of abundant amounts of type I IFNs in response to viral infections. Among conventional DC (cDC) populations, the CD8α+ DCs play a dominant role in the presentation of Ag in the context of viral infection, particularly to CD8+ T cells (1). CD8α+ DCs also can cross-present exogenous Ags on MHC class I molecules to activate CD8+ T cells (2) so do not necessarily require direct viral infection to initiate the CD8+ T cell response. In contrast, multiple DC subsets including CD8α− cDCs and CD4+ and double-negative (DN) DCs can play significant roles in stimulating CD4+ T cell responses (3, 4). This division of labor between DC subsets appears quite distinct in some viral infections, such as HSV-1 infection, and has led to the notion that DC populations are fundamentally specialized to handle either MHC class I or II pathogen-derived Ags. Despite this, in other infections, such as influenza infection, non-CD8α+ DC subsets also present abundant amounts of viral Ag to CD8+ T cells, implying that different infections can impact differently on DC populations in driving an immune response (5, 6). Most of our understanding of the induction of CD8+ T cell responses by DC subsets comes from the analysis of lytic infections. Temporal changes in the capacity of different DC subsets to present Ag in persistent infections have not been investigated in detail in vivo but are likely to play a key role in determining the capacity to rapidly induce an effective immune response.

Establishment of persistent infection by viruses such as γ-herpesvirus (γHV) is in part facilitated by immune modulatory proteins. These genes interfere with the expression of a variety of key components of the immune activation apparatus, including MHC class I and costimulatory molecules that are expressed on DCs (e.g., ICAM-1 and B7-2) (7). In the γHV family, the best characterized examples of viral genes that interfere with the expression of MHC class I glycoproteins on the surfaces of cells are Kaposi’s sarcoma-associated herpesvirus K3 and K5 (7) and the murine γHV-68 K3 (MK3) (8). Murine γHV-68 provides a useful model to study the host–pathogen interactions in a physiological setting. After intranasal infection, it establishes an acute lytic infection in the lung that is substantially controlled. Despite this response, the virus subverts the local immune response to establish lifelong persistence. The murine γHV K3 specifically inhibits MHC class I processing and in vivo limits the establishment of latency in spleen (9). This is achieved by directly degrading tapasin and TAP and through its action to ubiquitinate nascent H chains of classical MHC class I molecules, tagging them for proteasomal degradation (8, 10). Despite these effects, however, whether the virus targets particular cells types or tissues in vivo and where MK3 first exerts its action remain unclear. In earlier work, the deletion of MK3 from γHV-68 resulted in enhanced CD8+ T cell responses despite lower viral loads. It seems likely that DCs could be a major target of the effects of MK3 and that this would impact significantly on the development of the immune response if it occurred at an early stage of T cell priming. Such a strategy could result in failure to contain initial infection and facilitate systemic establishment of the virus.

In this study, we systematically investigated how a prototypical persistent virus, murine γHV, influenced the interplay between DCs and T cells during initial T cell priming. We found that one way the virus could diminish induction of the T cell response was to target early Ag presentation in different DCs to dampen rapid induction of the immune response. This early interference is likely to have broad implications for induction of effective pathogen immunity, particularly against persistent infections.
Materials and Methods

Mice

C57BL/6 (B6, H-2b), B6.SJL-PtpcrePep3bBoy/J (Ly5.1), OT-I (Ly5.2b), and Ly5.1OT-I mice were bred and maintained in specific-pathogen-free conditions at the animal facilities of The Walter and Eliza Hall Institute of Medical Research (Melbourne, Australia). Experiments with all of the mice began when mice were 6–10 wk of age and were approved by and performed in accordance with guidelines of The Walter and Eliza Hall Institute of Medical Research Animal Ethics Committees.

Generation of recombinant γHV viruses

The generation of MHV-68 engineered to express OVA from an intergenic expression cassette under a lytic cycle promoter has been described previously (11). This approach incorporates an ectopic copy of the 500 bp upstream of the MHV-68 M3 open reading frame (ORF) as a strong, ORF50-dependent lytic cycle promoter (12). The full-length OVA-encoding sequence was PCR-cloned between the M3 promoter and a bovine growth hormone poly-A site derived from pcDNA3 (Invitrogen, Carlsbad, CA). The expression construct was then subcloned into the MHV-68 genomic clone (75338–78717) and subcloned with its genomic flanks into pST76K-SR. This expression cassette was then recombined into the MHV-68 bacterial artificial chromosome (BAC) between the ends of ORF57 and ORF58. Infectious virus was reconstituted from BAC DNA by transfection into BHK-21 cells with Fugene-6 (Roche Diagnostics, Lewes, U.K.). The BAC cassette was removed by serial viral passage through NIH-3T3-CRE cells. Virus stocks were grown and titered on BHK-21 cells. All of the introduced mutations were confirmed as correct by viral DNA sequencing (11).

Generation of ΔMK3 viruses

The first recombinant MHV-OVA virus deficient in MK3 (ΔMK3SET) was generated by inserting a flippase (Flp) recombination target-flanked tetracycline-resistance gene into the MK3 ORF of an MHV-68 BAC (9). A derivative of this virus (ΔMK3TET+) was generated with Flp recombinase to remove the tetracycline-resistance gene, which left MK3 disrupted by a single Flp recombination target site plus a short flanking plasmid sequence (165 bp). This sequence interrupts the plant homedomain finger of MK3, rendering MK3 nonfunctional.

A second independent recombinant virus expressing OVA and lacking MK3 was generated using Cre-Lox technology. The MHV-68 K3 gene was disrupted on this background by digesting a SacI genomic clone (genomic coordinates 21383–28336) (13) with NruI (24851) and BsmI (24999). The BsmI-cut 5′-nt overhang was filled in with Klenow fragment DNA polymerase (New England Biolabs, Hitchin, U.K.), and the two blunt ends were ligated together. The mutant SacI clone was then subcloned into the SacI site of pST76K-SR and recombined into the MHV-OVA BAC by standard methods (14). Infectious viruses were reconstituted by transfecting BAC DNA into BHK-21 cells with Fugene-6. The loxp-flanked cassette was removed by virus passage in NIH-3T3-CRE cells. All of the viruses were grown in BHK-21 cells. Infectious cultures were cleared of infected cell debris by low-speed centrifugation (1000 × g; 3 min). Virus titers were determined by plaque assay on BHK-21 cells (15). The structures of all of the recombinants were checked by Southern blot analyses and by restriction enzyme mapping of BAC DNA. MK3 also was sequenced in each recombinant to confirm the exact insertion site (data not shown). Growth curves of the first recombinant MHV-OVA virus deficient in MK3 (ΔMK3TET+) was generated with Flp recombinase to remove the tetracycline-resistance gene, which left MK3 disrupted by a single Flp recombination target site plus a short flanking plasmid sequence (165 bp). This sequence interrupts the plant homedomain finger of MK3, rendering MK3 nonfunctional.

Virus infections

Mice were infected intranasally with 3 × 10^5 PFU of recombinant MHV-OVA (designated MK3Δ-OVA) or ΔMK3-OVA in 25 μl PBS under brief halothane anesthesia.

Preparation OVA-specific CD8+ transgenic T cells and CFSE labeling

Peripheral lymph nodes (LN; inguinal, axillary, brachial, sacral, and superficial cervical) were obtained from Ly5.1OT-I C57 B16 TCR transgenic mice and purified using a mixture of optimally titered Abs to deplete cells expressing CD11b (Mac-1, M1/70), Mac-3 (F4/80), Ter-119, Gr1 (RB6-8c5), MHC class II (M5/114), and CD4 (GK1.5) followed by sheep anti-rat magnetic beads (Dynabeads; Dynal, Oslo, Norway). Enriched cells contained 87–96% specific TCR transgenic T cells. In some experiments, these cells were then labeled with CFSE (Molecular Probes, Eugene, OR) by incubating 10^7 purified cells per milliliter with 5 μM CFSE for 10 min at 37°C. Cells were then washed three times in HEPES-modified Eagle’s medium containing 2.5% FCS.

Isolation of DCs from LN and spleen

DCs were isolated using collagenase/DNase and EDTA to disrupt T cell–DC complexes. Cells not of the DC lineage were depleted by incubating in predetermined optimal concentrations of purified Abs anti-CD3 (KT3), anti-Thy1 (T24/31.7), anti-CD19 (ID3), and anti-erythrocyte (TER-119) and then removing the Ab-binding cells with anti-rat IgG-coupled magnetic beads (Dynabeads) (16, 17). The DCs in the enriched populations were gated as CD11c+ cells before being sorted into specific subsets by FACS (MoFlo instrument; DakoCytomation, Fort Collins, CO). For LNs, DCs were sorted into CD8α+, CD45RA−, and DN DCs or, alternatively, CD8α− (conventional CD8α+ DCs), CD8α−CD11b+, and CD11b+ DCs. Postsorting analysis of sorted DC populations showed that purity ranged from 90 to 99% and that the average purity of the populations used for stimulation was 98%.

Analysis of in vitro proliferation of naive T cells by DCs

A total of 5 × 10^5 enriched CFSE-labeled OT-I CD8+ TCR transgenic cells were added to graded numbers of flow cytometrically sorted DCs in 200 μl RPMI 1640 containing 10% FCS, 50 μM 2-ME, 2 mM L-glutamine, 100 μM penicillin, and 100 μg/ml streptomycin in 96-well V-bottom plates (Costar, Corning, NY). Cultures were analyzed for proliferation after 6 h. Cells were harvested and stained with anti-CD8α-allophycocyanin (33-6-7; BD Pharmingen, San Diego, CA) and resuspended in 150 μl flow cytometry buffer containing 2.5 × 10^5 blank calibration beads (6 μm; BD Biosciences, San Jose, CA). Samples were analyzed by flow cytometry by Pe− exclusion until 5 × 10^3 to 1 × 10^4 beads were collected.

Ag presentation assays

DCs for in vitro analysis of Ag presentation were grown from bone marrow progenitors of C57BL/6 mice in RPMI 1640 with 10% FCS, 50 μM 2-ME, 100 U/ml penicillin, 100 μg/ml streptomycin, and 7.5 ng/ml GM-CSF (PeproTech, Rocky Hill, NJ). To assay MHC class I-restricted presentation of the H2-Kb-restricted OVA epitope, DCs or peritoneal macrophages (2.5 × 10^5 cells per well) were infected or not with MK3Δ-OVA or ΔMK3-OVA (3 PFU per cell, 4 h) and then incubated with a lacZ-expressing OVA-specific T cell hybridoma (9). After 18 h of culture, the cells then were washed in PBS and lysed in PBS containing 5 mM MgCl2, 1% Nonidet P-40, 0.15 mM chlorophenol-red-β-galactosidase (Merck Biosciences, Nottingham, U.K.) to assay β-galactosidase activity. After 2–4 h, the absorbance at 595 nm was read on a Benchmark microplate reader (Bio-Rad, Hercules, CA).

In some experiments, direct analysis of MHC class I/OVA complexes was detected by incubating FMS-like tyrosine kinase 3 ligand–ganged (100 ng/ml for 6 in vitro [rFMS-like tyrosine kinase 3 ligand; R&D Systems, Minneapolis, MN]) bone marrow–derived DCs with biotinylated mAb 2b-D1 (Jomar Bioscience, Kensington, South Australia) (18) together with CD11c+ followed by streptavidin-PE. The mAb 2b-D1 recognizes K8/SIINFEKL MHC class I complexes on the surfaces of cells. On day 5 of culture, FLT3L-stimulated DCs were infected overnight with MK3Δ-OVA, ΔMK3-OVA, or MHV-68 (3 PFU per cell) prior to staining. Samples were analyzed by flow cytometry by using Pe− exclusion to exclude dead cells.

Clodronate liposome treatment

Depletion of inflammatory Gr1+ monocytes was performed according to the protocol previously described (19). Briefly, liposome-encapsulated clodronate (dichloromethylene bisphosphonate) was a gift from Roche Diagnostics, Mannheim, Germany) was prepared as described previously (20). To maintain depletion of monocytes throughout the infectious period, 200 μl clodronate-loaded liposomes were administered i.v. commencing 1 d prior to infection and continuing every other day for the duration of the experiment.

Statistical analysis

Statistical comparisons were made on all of the data using the Student two-tailed t test. All of the experiments contained two to five replicates per experimental parameter. Statistically significant comparisons, p < 0.05, are denoted with an asterisk.

Results

Herpesvirus evasion protein MK3 limits maximal Ag presentation and priming of CD8+ T cells early in infection

The activation of T cells during a localized infection occurs in the draining LNs where the bulk of T cell priming to a primary infection is thought to occur (21). In an infection, this represents the first point of contact between cells carrying viral Ag and virus-
specific T cells that are critical to the local control of infection. Murine γHV infection provides a robust model to examine how the interactions between DCs and T cells are affected by a virus encoding a gene known to disrupt Ag presentation. To facilitate detailed tracking of T cell–DC interactions and the influence of MK3 in vivo, we used a γHV encoding OVA (MK3+-OVA) and generated complementary recombinant viruses in which the OVA protein was inserted into the K3 ORF of a γHV-68 BAC (Fig. 1). This approach disrupts the plant homeodomain finger of MK3, rendering MK3 nonfunctional (9).

Persistent viruses, in contrast to acute viruses, commonly infect DCs, and modulation of DC function may offset the opportunity to prime T cells (22). Earlier work has suggested that MK3 was not active in DCs during infection (23). More recent evidence showed that MK3 can operate in DCs, at least in vitro (24), but it is less clear that it is active in vivo. We hypothesized that the enhanced CD8+ T cell response generated in the absence of MK3 (9) occurred by targeting MHC class I molecules in various types of APCs shortly after infection. To test this in an in vivo system in which we could trace Ag-specific presentation, we looked for the capacity of different APC populations to present OVA Ag encoded by competent or MK3-disrupted γHV. DCs and peritoneal macrophages were isolated from mice and then infected in vitro with either MK3+-OVA or ΔMK3-OVA virus (5 PFU per cell). A lacZ-encoding OVA-specific T cell hybridoma was used to detect OVA-specific MHC class I Ag presentation (25). Infection with the ΔMK3-OVA virus was accompanied by a ∼2-fold increase in the level of Ag presentation in both types of APCs (Fig. 2A, upper panel). Similarly, in vitro-generated DCs infected with ΔMK3-OVA showed an increase in both the number and the mean fluorescence intensity of CD11c+ cells expressing the MHC class I Kb/SIINFELK complexes compared with those of MK3+-OVA-infected DCs (Fig. 2A, lower panel). To establish if the effect of MK3 also translated to in vivo-derived DCs and priming of Ag-specific CD8+ T cells, we assessed the capacity of virally infected DCs to drive amplification of naive CD8+ T cells (Fig. 2B). DCs were enriched directly from spleens of naive C57BL/6 mice and then infected with either MK3+-OVA or ΔMK3-OVA viruses. The proliferation of naive OT-I cells was assessed following 60 h of in vitro culture. Increased expansion of OVA-specific T cells was observed when they were stimulated with ΔMK3-OVA-infected DCs, where the MK3 gene has been disrupted, and therefore MHC class I Ag presentation should proceed normally (Fig. 2B). The diminished presentation in MK3+-OVA infection could not be attributed directly to differences in DC death because
the expression of annexin V in DCs was similar in both infections (data not shown). These data indicate that MK3 is functional in both in vitro-derived and purified in vivo DCs and that it can modulate the priming ability of the exposed DCs following in vitro infection.

Induction of the immune response to murine γHV depends almost exclusively on DCs to activate naive CD8+ T cells (26). Similarly, in the setting of ΔMK3-OVA virus infection, CD11c+ cells were necessary for T cell proliferation for both MK3+-OVA and ΔMK3-OVA herpesviruses (Supplemental Fig. 1), demonstrating that this requirement for DCs in T cell priming was not altered in vivo by truncation of the MK3 gene. Analysis of lung viral titers in C57BL/6 mice suggested that viral replication in vivo was similar for the

**FIGURE 2.** Diminished amplification of OVA-specific T cells in the presence of γHV K3. A, Upper panel. H2b DCs or peritoneal macrophages were infected with MK3+ or MK3− viruses as indicated for 4 h, washed, and incubated overnight with a T cell hybridoma that recognizes the SIINFEKL epitope of OVA. Each hybridoma produces β-galactosidase on activation and was measured with chlorophenol-red-β-D-galactopyranoside. Mean ± SD absorbance values of triplicate cultures are shown. The data show one of three equivalent experiments. Lower panel. In vitro-generated DCs were infected with MK3+-OVA, ΔMK3-OVA, or MHV-68 (lacking OVA) viruses overnight, then stained with anti-CD11c mAb and Kβ/SIINFEKL mAb 25-D1. Profiles are gated on CD11c+ cells and are representative of two independent experiments with similar results (n = 6 individual cultures). Gray line, MHV-68; black line, MK3+-OVA or ΔMK3-OVA, respectively. B, DCs were purified from the mediastinal LNs of naive C57BL/6 mice by depletion of non-DC lineages. DCs were pulsed in vitro with 5 PFU per cell of either MK3-OVA or MK3-OVA viruses for 45 min at 37˚C, then washed twice with complete media. Titrating numbers of infected DCs were cultured with 5 × 10^5 CFSE-labeled OVA-specific CD8+ T cells. Proliferation was analyzed at 60 h of culture. Data are pooled from two independent experiments performed in triplicate and show the mean ± SEM. C, Schematic representation of the experimental approach to in vivo analysis of Ag presentation soon after infection. C57BL/6 mice were infected with MK3+-OVA or ΔMK3-OVA at −5, −4, −3, −2, −1, or day 0, then 1 × 10^6 purified congenically marked CFSE-labeled Ly5.1+ OVA-specific CD8+ T cells were adoptively transferred into all mice on day 0. The mediastinal LNs were analyzed from individual mice 3 d after transfer, and the extent of proliferation of OT-I cells was determined by flow cytometry. D, Enumeration of Ly5.1+OT-I CD8+ T cells that had undergone proliferation in the draining mediastinal LN 72 h after transfer was assessed by flow cytometric analysis of the loss of CFSE staining. Data are pooled from two (day 5) to three (days 1–4) independent experiments and show the mean ± SEM of four to seven mice at each time point. *Statistically significant differences, p < 0.05. E, Representative CFSE proliferation profiles of OVA-specific CD8+ T cells transferred into mice 1–5 d after intranasal infection with either MK3+-OVA or ΔMK3-OVA viruses.
different viruses (Fig. 1E, right panel) (9). Next, we determined whether changes in Ag presentation mediated by MK3 affected the induction of virus-specific CD8+ T cell proliferation in vivo (Fig. 2C–E). After γHV infection, we adoptively transferred congenically marked CFSE-labeled Ly5.1+ OVA-specific CD8+ transgenic T cells into infected recipient mice at various days postinfection (Fig. 2C). In this setting, the expression of MK3 in wild-type MK3-OVA virus consistently reduced the overall capacity of the virus to stimulate naive OT-I cells compared with that of the MK3-disrupted virus (Fig. 2D, 2E). This effect was most obvious during the first 4–5 d of infection and showed a delayed induction in the capacity of mice infected with MK3+-OV A (MK3+) but not ΔMK3-OVA (MK3–) to rapidly induce optimal expansion of Ag-specific CD8+ T cells during priming. Thus, MK3 appears to affect the ability of APCs to generate maximal antiviral CD8+ T cell responses during the crucial early priming phase of infection by targeting DC populations (Fig. 2).

Deletion of MK3 function uncovers Ag presentation by CD11b+ DCs

Although MK3 transcripts are detected in DCs during the establishment of latency (27), the impact of MK3 on DCs during acute in vivo infection is unclear. Most of our understanding of the function of MK3 is derived from in vitro studies (23, 24). In this setting, lytically infected DCs downregulated CD86 and MHC class I expression, resulting in poor Ag presentation to CD8+ T cells (24). To understand how MK3 induces impairment of CD8+ T cell expansion in vivo, we explored the possibility that Ag presentation by DCs that first traffic from the infected lung and encounter CD8+ T cells was negatively regulated by the expression of MK3. To address this, we isolated DC subsets from the draining mediastinal LN 3 and 5 d after intranasal infection with either MK3+-OVA or ΔMK3-OVA. The DCs within the DN subset are composed of multiple subsets (including plasmacytoid DCs, trafficking DCs, and LN-resident DCs), and these populations appear to have distinct functions in other viral infections (5, 6). Mediastinal LN DCs were depleted of plasmacytoid DCs and then divided into lung-derived CD8α–CD11b– trafficking DCs, LN-resident CD8α+CD11b– DCs, and CD8α+CD11b+ DCs (Supplemental Fig. 2A). In this setting, CD11b– DCs express the αβ integrin CD103, whereas CD11b+ DCs lack expression of CD103 (Supplemental Fig. 2B). Purified DCs then were cultured for 60 h with 5 × 10⁴ naive CFSE-labeled OT-I cells. With this approach, it became apparent that MK3+-encoded OVA Ag was presented by at least two populations of DCs within the mediastinal LN, namely, CD8α+ DCs and CD8α–CD11b– DCs (Fig. 3). This sorting regimen revealed that, in the absence of MK3, the CD8α+CD11b+ DCs also played a key role in driving the proliferation of naive OVA-specific CD8+ T cells (Fig. 3). Conversely, in the presence of MK3, only very weak Ag presentation was detectable in this population. These data show that MK3 interferes with overall Ag presentation but can specifically interfere with particular subsets, namely, the CD11b+ DCs, resulting in a dampening of the key priming phase of the T cell immune response.

**FIGURE 3.** Fine dissection of DC populations uncovers subset-specific MK3 inhibition of Ag presentation in vivo. Naive C57BL/6 mice were infected with 3 × 10⁴ PFU of MK3+-OVA (A, C) or ΔMK3-OVA (B, D). Three and five days postinfection, Ag presentation of different DC subsets was assessed. The mediastinal LNs were isolated at 3 (A, B) and 5 d (C, D) after intranasal infection. CD11c+ DC subsets were purified by flow cytometric sorting into CD8α–CD11b–, CD8α+CD11b–, and CD8α+CD11b+ DCs (Supplemental Fig. 2A) before culturing titrating numbers of each subset with 5 × 10⁴ CFSE-labeled OVA-specific CD8+ T cells for 60 h. A and B show the mean ± SEM of four independent experiments; C and D show one of three similar experiments. E, Mediastinal LNs were pooled from 15–20 mice at each time point, and DC subsets were purified by flow cytometric sorting after depletion of non-DC populations. A total of 5 × 10⁴ purified cDCs subsets were cultured with 5 × 10⁴ CFSE-labeled OVA-specific CD8+ T cells. Proliferation was analyzed at 60 h of in vitro culture. Data show representative profiles of one of three similar experiments.
MK3 expression impairs recruitment of conventional and inflammatory DCs to the lung-draining LN

Recently, inflammatory monocyte-derived DCs (moDCs) have been implicated in Ag presentation during the immune response (28). This subset expresses intermediate levels of the classical DC lectin CD11c together with high levels of the adhesion molecule CD11b and Gr1, whereas cDCs can be distinguished from these cells by their high levels of CD11c (Figs. 4, 5) (19). To determine the involvement of inflammatory monocytes in γHV infection, we first compared the number of cDCs and inflammatory moDCs recruited into the mediastinal LN following MK3+–OVA and ΔMK3–OVA infection (Fig. 4). In this setting, we found a reduction in both cDCs and moDCs when MK3 was expressed. This difference was most striking for moDCs and suggested that they could be involved in virus-specific T cell activation (Fig. 4). To test this, we determined the capacity of moDCs isolated from mediastinal LNs of infected mice (Fig. 5A) to induce proliferation of naive OVA-specific CD8+ T cells (Fig. 5B). cDCs were able to effectively activate CFSE-labeled OT-I CD8+ T cells using this approach, whereas moDCs showed no apparent capacity to induce the proliferation of virus-specific CD8+ T cells. To test this further, we used an in vivo model where moDCs are not subjected to ex vivo manipulation prior to determining their functional ability. γHV-infected mice were depleted of moDCs using clodronate-loaded liposomes, then congenically marked OVA-specific CD8+ T cells were adoptively transferred into mice, and their proliferation was assessed (Fig. 5C, 5D). This approach has been used previously to selectively remove moDCs in vivo for assessment of Ag presentation (29). Despite the markedly enhanced recruitment of moDCs that we observed in the absence of MK3 (Fig. 4), the expansion of OVA-specific CD8+ T cells was not significantly different in mice depleted, or left undepleted, of moDCs for either K3+ or K3− γHV infection (Fig. 5D). Thus, we found little evidence for a role for moDCs in early Ag-specific priming of CD8+ T cell responses after γHV infection (Fig. 5B, 5D). The full implications of the alteration of the APC recruitment pattern are yet to be determined, but these data imply that additional factors associated with viral infection can secondarily influence optimal DC recruitment, leading to an impaired or delayed immune response.

Discussion

A hallmark of persistent infections is the failure to develop protective immune responses sufficiently rapidly to prevent dissemination of the pathogen throughout the body. In several infections such as HIV (30), hepatitis virus (31) and Mycobacterium tuberculosis (32), there is a significant delay between pathogen infection and induction of the adaptive immune response. This lag period potentially allows uncontrolled growth and/or dissemination of the pathogen. Although high antigenic variability, slow replication, poor immunogenicity, and the capacity of the pathogen to disrupt key recognition elements of the immune system have been causally implicated, the specific mechanisms underpinning this lag period have been poorly elucidated (30). It is beginning to emerge, however, that pathogens can delay induction of an immune response by sequestration of organisms as a strategy to establish persistence (32). In the case of M. tuberculosis infection, although bacteria were present in the lung, initiation of the immune response could not proceed due to a lack of DCs expressing mycobacterial Ags in the mediastinal LN despite normal lung to LN migration by these cells (32).
Previously, γHV MK3 has been shown to interfere with the establishment of viral latency in the spleen, but the cells involved in this process and the tissue site of action in vivo have been unclear (9). We show that in persistent γHV infection this can occur in DCs in the lung-draining LNs. Furthermore, disruption of MHC molecules in DCs by MK3 early postinfection limits amplification of T cells. Indeed, the overall capacity of DCs to present Ag to T cells is a critical parameter to optimize antiviral responses. The effects of MK3 diminished overall Ag presentation but, in addition, appeared to ablate presentation by CD11b+ DCs early in infection and to limit recruitment of moDCs into the immune response. These findings establish a setting for suboptimal induction of early γHV T cell immunity in the lung-draining LN mediated at least in part by MK3 targeting or impairing one or more DC subsets. The broader reliance on non-CD8α DC subsets identified in this study is concordant with other lung infection models where it is now clearer that multiple DC subsets are necessary for full immune induction rather than this being limited to a single DC population (such as CD8α+ DCs) (5, 6, 33).

In the case of MK3−/−OVA infection, although CD8α+ DCs did present significant Ag during the first few days of infection, the effect of MK3 was most striking within the non-CD8α DC subsets where elimination of MK3 uncovered the ability of an additional subset of DCs (the CD8α+/−/CD11b+ DCs) to present viral Ag. It is possible that efficient early Ag presentation by CD8α+ DCs may be associated with the secreted nature of the surrogate Ag OVA facilitating cross-presentation of Ag. As the virus replicates, however, it is likely that a broader number of DCs are directly infected and thus may be negatively influenced by viral evasins acting to downmodulate the induction and overall magnitude of this early priming response in the regional LN. These effects open the way for a pathogen to largely negate early detection by the immune system while it establishes the foothold that is the basis of persistence.

An additional unexpected finding of this study was the alteration in recruitment of inflammatory moDCs detected in the mediastinal LN. The precise role played by moDCs in infection is currently unclear, although they have been implicated as main producers of TNF-α and inducible nitric oxide synthase and may play a role in Ag presentation (28, 29). In MK3−/−OVA infection, moDCs do not effectively present MHC class I viral Ag and thus do not appear to be directly involved in the early phase of the induction of the CD8+ T cell response. It has been proposed that moDCs may be principally involved in modulating the innate immune response during inflammation and thus play essential functions during infection.
although the precise nature of this remains to be elucidated. Despite this, the fact that Ag presentation of DC subsets and recruitment of moDCs are both modulated by the action of MK3 during MK3-ova infection highlights the multiple pathways that may be influenced by such an evasion gene. This opens the possibility that in persistent infections differential effects on DC subsets by the virus itself may facilitate the establishment of persistence by allowing it to sneak through the frontline defense in the LN early in infection (34).

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Disclosures

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