Kinetics of Major Histocompatibility Class I Antigen Presentation in Acute Infection

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J Immunol 2009; 182:902-911; doi: 10.4049/jimmunol.182.2.902
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Ag presentation within the regional lymph node is crucial for the initiation of CD8+ T cell responses following viral infection. The magnitude and quality of the CD8+ T cell response are regulated by the interplay between the size of the APC population and duration of Ag presentation. To understand how these parameters are finely regulated during an immune response, we have investigated the dynamics of Ag presentation in influenza A virus and HSV-1 infection. In both infections, APC production was calculated to occur over the first few days of infection, after which there was slow exponential decay over a period of up to 2 wk. This production rate is most likely determined by the Ag availability and recruitment and/or maturation rate of dendritic cells. APC production was found to closely parallel lymph node cell recruitment in both infections. This was greatest in the first 6 h of infection for HSV and over the second and third day for influenza. In HSV infection, the peak production also coincides with peak viral levels. By contrast, in influenza infection, APC production ceased between the third and fourth day despite the presence of high levels of virus until 5 days after infection. These analyses demonstrate that two quite different self-limiting infections generate the APC necessary to drive T cell responses early in infection at different rates. Understanding how such contrasting kinetics of Ag presentation impacts on the growth and size of developing protective T cell populations has important implications for the design of vaccines and immunotherapies. The Journal of Immunology, 2009, 182: 902–911.

P rotective immune responses against pathogenic infections depend on the amplification of rare naive Ag-specific CD8+ T cells that differentiate to produce effector and memory T cell populations that play a key role in controlling pathogenic infections. The initiation of a CTL response requires the interaction of naïve CTLs with specialized APC (such as dendritic cells (DC)) bearing their cognate Ag (1–4). The process by which the number, type, and quality of APC regulate the tempo of the immune response following antigenic challenge is critical to understanding how the overall sizes of T cell populations are generated. Despite this, the kinetics of Ag presentation and the factors that influence the production and loss of APC remain poorly understood. At steady state, DC are found in an immature state and have a short half-life, lasting only 2–3 days (5, 6). In this state, immature DC rapidly and efficiently load both exogenously derived (cross-presented) and endogenously derived (e.g., derived from pathogen replication) Ags onto MHC molecules for presentation to presentation to T cells (7, 8).

Following pathogen infection, DC are exposed to a myriad of inflammatory signals, particularly cytokines, that could influence their capacity to effectively signal to T cells. The response to tissue damage from the infection and the inflammatory milieu is collectively indicated by the initiation of the DC maturation program characterized by the rapid export of intracellular stores of MHC class I and II molecules to the cell membrane and the expression of costimulatory molecules such as CD80, CD86, and CD40 (9–13). These changes in DC are accompanied by down-modulation of further loading of exogenous, but not endogenous, MHC class I and II Ags, so that presentation reflects the antigenic experience of the cell before full activation (14, 15).

Long-term Ag presentation appears to be a feature of primary immune responses (16–18). This may be essential to ensure the formation of the stable T cell-DC interactions necessary for activation, proliferation, and differentiation of T cells. It may also allow recruitment of a diverse array of rare naïve T cells throughout the immune response (19–21). Migration of activated T cells from the lymph node (LN) to the site of infection, as they acquire effector function, appears to limit the potential cytotoxic effects of CTL on DC in the LN (22, 23). This mechanism, synergized with the induction of molecules such as Spi-6 that can blunt the cytotoxic effects of lytic granules, could inhibit APC killing as an immediate consequence of DC-T cell interactions (22). Thus, prolonged Ag presentation could require either the extended survival
of DC (24) or the continued recruitment of DC as they enter lymphoid tissues draining the site of infection (either by continued maturation of DC, continued infection of DC, or the transfer of pathogen-derived Ags to progeny DC arising from dividing precursors within the lymphoid tissues (25, 26)).

In contrast to primary immune responses, secondary immune responses are characterized by the loss of DC by CD8-mediated mechanisms (23, 25, 27, 28). These mechanisms are likely to play an important role in shaping the CD8$^+$ T cell immune response. For example, prevention of persistent activation of CTL may limit immunopathology that could be mediated by pre-existing CTL that express potent effector function (23, 29, 30). This regulation of CTL would serve as an important negative feedback mechanism precisely regulating T cell expansion (27).

The rapid activation of virus-specific CD8$^+$ T cells is crucial to ensure effective control of infection. Following s.c. HSV-1 infection, CD8$^+$ T cell response is initiated within 2 h after infection (31, 32). In contrast, in SIV infection in monkeys, the delay of some 10 days before the CD8$^+$ T cell response is initiated provides an opportunistic window allowing uncontrolled growth of the virus and the subsequent establishment of persistent infection (33, 34). A similar delay in T cell activation is observed in tuberculosis infection in mice (35). This wide variation in the timing of CD8$^+$ T cell activation suggests significant differences in the developmental program of initial Ag presentation. At present, little is known about the rate and timing of Ag presentation during early infection.

Although it is widely appreciated that factors regulating the production and death of DC must play an important role in regulating CD8$^+$ T cell responses in pathogen infection and in maintaining homeostasis of immune responses, the mechanisms regulating this process are currently poorly understood. Elegant approaches to manipulate the duration of Ag presentation have provided insight into how the duration of Ag presentation influences the size of the T cell response. For example, the duration of Ag presentation has been curtailed by truncating the period of bacterial infection with antibiotics (36), or removal of the infectious site in viral infection (17). Conversely, the synergistic coexpression of Ag with molecular regulators of apoptosis, such as Bcl-x$_L$, prolonged DC survival and increased CD8$^+$ T cell expansion, resulting in immunity or autoimmunity (37, 38). These studies imply that the T cell stimulation in vivo is not a simple on/off switch dictated by the presence or absence of APC. Instead, the T cell stimulation at any time will vary according to the number of APC available. In a physiological response, such as that to a pathogen, it is likely that the overall magnitude and duration of T cell expansion during an infection are a balance between multiple factors, including the timing, duration and rate of APC production, antigenic load of the APC, and the rate of decay of APC.

In this study, we have investigated the kinetics of Ag presentation during primary infection following intranasal infection with influenza A (flu) virus and s.c. infection with HSV to extend our understanding of how these features of the immune response contribute to both the initiation and control of T cell amplification (23, 32, 39). Viral loads, the number of LN cells, and number of APC were measured at regular intervals following infection to allow us to model the dynamic regulation of Ag presentation during acute infection.

Materials and Methods

The present manuscript describes the analysis and modeling of data combined from a number of sources: the experimental methods and data on APC numbers, LN cell numbers, and viral loads following HSV-1 infection were previously reported elsewhere (17, 32, 39). The experimental data on APC numbers, LN cell numbers, viral loads, and respiratory DC numbers were largely from our previously published work (23), but also include our additional unpublished data from current experiments. The methods are detailed in the original publications and briefly outlined below.

Mice

C57BL/6 were obtained from The Walter and Eliza Hall Institute of Medical Research for influenza experiments, as previously described (17), or from Department of Microbiology and Immunology, University of Melbourne (23, 32), and Monash University for HSV experiments (39). They were maintained under specific pathogen-free conditions. Experiments with mice began when they were between 6 and 10 wk of age.

Virus infections

Mice were anesthetized with methoxyflurane and then intranasally infected with a nonlethal challenge of 10$^5$ PFU of A/HKX31 (H3N2, HKX31) influenza virus diluted in 25 μl of sterile PBS. For HSV-1 infections, mice were inoculated in the footpad with 4 × 10$^5$ PFU KOS strain HSV-1.

Detection of Ag presentation by LN DC

APC were isolated essentially as described (16, 40). Briefly, the regional draining LN (medialittal or popliteal LN) were digested for 20 min at room temperature with collagenase/DNase and then treated for 5 min with EDTA to disrupt T cell/DC complexes. Previously, we have shown that depletion of cells expressing CD11c removed Ag-specific stimulatory capacity, indicating that Ag presentation was limited to CD11c$^+$ DC (16, 40). The LacZ-inducible hybridomas specific for D$^b$ nucleoprotein (NP)866–874, D$^d$ acid polymerase (PA)224–233 (BWZ-IFA.PA1), and K$^b$ glycoprotein B (gB)498–505 (HSV-2.3.2E2) (32) were maintained in medium containing 10% FCS, 50 μM 2-ME, 2 mM l-glutamine, 100 U/ml penicillin, and 100 μg/ml streptomycin (hybridoma medium). These clones were used to analyze Ag presentation by LN cells, as previously described (16, 32, 40).

Data analysis

Linear regression analysis of APC decay. To understand the duration of Ag presentation in vivo, we analyzed the decay of APC following their peak assuming simple exponential decay. The decay constant (δ) of the number of APC was estimated with linear regression (Pearson) of the experimental data on APC numbers, LN cell numbers, and viral loads following HSV-1 infection (31, 32). In contrast, in SIV infection in monkeys, the decay slopes were compared for the different epitopes using Prism 5.01 (GraphPad). Data points in which no LacZ$^+$ cells were observed were taken to be half the detection threshold and were only included in the analysis when they were lower than the regression line through the data. Analysis was performed after the peak in data. This was from day 3 onward in influenza infection, and after day 2 postinfection for HSV. To decay slopes were compared for the different epitopes using Prism 5.01 (GraphPad). Slopes are reported as the least squares fit coefficient along with the SE.

Calculation of APC production rate

The geometric mean of experimental data for APC numbers was used to calculate the APC production rate based on the differential equation shown in Equation 1. APC decay is modeled with a constant exponential decay rate (δ) and the increase in APC presenting Ag (A(t)) is embodied by the production rate (ρ).

\[
\frac{dA(t)}{dt} = \rho(t) - \delta A(t)
\]

The APC data were piecewise modeled over each time interval in which data existed. This interval in sampling had a range from 2 h to 1 day. Over these intervals, production was considered to be constant, allowing a general solution for Equation 1 to be obtained. An expression for the production over each interval can thus be written as follows:

\[
\rho(t) = \frac{\delta(A(t) - A(0)e^{-\delta t})}{1 - e^{-\delta t}}
\]

In the above equation, A(0) is the geometric mean APC level at the start of the time interval, A(t) the value at the end of the interval, and t is the length of the interval. The decay constant (δ) was set to the value determined from linear regression analysis of the APC decay data to calculate the production rate. Because this linear regression analysis does not take production into account (and thus underestimates the true loss rate of APC), this implies that the calculated production rate is a minimal estimate. APC decay was considered strictly exponential; therefore, any negative

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specific for the immunodominant peptides derived from NP (DbNP366–374, PA (DbPA224–233, KbgB498–505), or gB (KbgB498–505) recognized by CD8\(^+\) T cells. Data for each viral infection are pooled from individual responses from two to five experiments. Data points with a square around them indicate where no LacZ\(^+\) cells were detected, and the value set to half the detection threshold. Lines indicate best fit exponential decay slopes.

values calculated for \( \rho \) were considered to be an artifact and subsequently set to an arbitrarily small number (1 APC/day). The production rates shown are based on the mean APC number for each time point. As illustrated in Fig. 1, there was both variation in levels around this mean for each time point, as well as variation in the number of samples for each time point. We took advantage of this variation to calculate confidence intervals on the production rate by bootstrapping (see below).

**Modeling APC dynamics**

The differential equation for the model (Equation 6) was solved using Matlab’s ode45 function. Parameters were then optimized with fmincon by minimizing the sum of squares log error.

For the model described by Equation 6, the rate of increase in LN cell population was calculated for the flux of cells. The flux term (\( \phi \)) is therefore expressed as follows:

\[
\phi(t) = \frac{dL(t)}{dt} \tag{3}
\]

The rate of change in LN cell population is calculated as the rate of change between pairs of geometric mean values. Log-linear interpolated viral load data were calculated from the geometric mean viral load as exponential growth and decay were assumed. Negative values were set to zero.

The model used data on lung-derived DC (LDC) (Equation 7) used data from CFSE-labeled DC from the lung to characterize the production rate of APC. CFSE was administered 6 h before infection (23, 41).

**Statistics**

To estimate the confidence intervals for the parameter estimates in the models, we used a bootstrapping approach. Because LN cell number and APC number were obtained in the same animal, we drew this paired data to recalculate LN cell number or dL/dt for each bootstrap sample. Data were sampled with replacement from the original dataset, and 500 bootstraps were performed. Mean values for \( k \) and \( \delta \) and their distribution were obtained from the bootstrap simulations. Because the number of parameters in each of the models is the same, it is possible to compare models in terms of the sum of squares error of the log-transformed data (as used for optimization). Data are presented as mean ± SDs, and Prism 5.01 (GraphPad) or Matlab (The MathWorks) was used for all the statistics and simulations.

**Results**

To gain insight into how APC are dynamically regulated during self-limiting pathogen infections, we examined the kinetics of generation and loss of APC during either influenza virus or HSV-1 infection. This approach focused on analysis of APC presenting the immunodominant CD8\(^+\) T cell epitopes H-2D\(^b\)NP366–374 and H-2D\(^b\)PA224–233 derived from influenza virus NP and PA, and H-2K\(^a\)gB498–505 derived from herpes simplex-1 gB.

Although DC are normally considered to be pivotal initiators of the immune response through their interactions with naive or memory T cells (42–44), there is a growing recognition that the regulation of DC during the effector phase of the immune response can be critical in determining the overall expansion of CD8\(^+\) T cells (27).

**Decay of APC in vivo**

Our initial studies of the dynamics of APC regulation in vivo suggested that the loss of APC after primary infection followed simple exponential decay, whereas APC production may be more complex (23). Therefore, we first addressed the kinetics of APC loss (described by the decay rate constant) before addressing the more complex factors influencing APC production (below). We analyzed the decay of APC following their peak by assuming simple exponential decay and performing linear regression analysis on the log-transformed data for APC number over time (Fig. 1). The decay rate constants of APC for the different epitopes are shown in Table I, as well as the estimated t\(_{1/2}\) of the APC. We note that because these rates do not consider any APC production after the peak, they are thus minimum estimates of loss rate.

The decay slope of APC number for APC presenting the D\(^b\)-NP epitope and D\(^b\)-PA epitopes during the primary response to influenza infection were previously analyzed (23) and were not found to be significantly different from each other (0.88 ± 0.03 day\(^{-1}\) for NP, and 0.82 ± 0.09 day\(^{-1}\) for PA (F = 0.831 for n = 108 degrees of freedom; \( p = 0.36 \)). Importantly, the decay appeared remarkably uniform over time (e.g., from day 3 to 15 in the case of NP; Fig. 1), and appeared not to depend directly on the number of CTL or CD8\(^+\) T cell-dependent lytic mechanisms (23).

To determine whether the patterns identified in APC for influenza virus infection extended more generally to other infections, we expanded our analysis to examine the loss of gB-presenting APC following HSV\(^+\) infection. The previously published kinetics of APC in the draining popliteal LN were carefully enumerated following footpad infection using the gB-specific lacZ\(^+\) T cell hybridoma (17, 32). The decay slope of APC presenting the HSV-gB epitope (0.46 ± 0.03 day\(^{-1}\)) was significantly different from that of either influenza virus epitope (\( p < 0.0001 \) for comparison with both NP and PA). Consistent with the decay of APC expressing influenza-derived epitopes, the decay of the cells presenting the HSV-gB epitope appeared remarkably uniform over time (Fig. 1).

The constant exponential decay of in vivo Ag presentation observed in these experiments suggests that studies aimed at identifying the duration of Ag presentation may be problematic. The

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**Table I. Estimation of decay rate constant for NP/PA and gB epitopes following viral infection**

<table>
<thead>
<tr>
<th>Virus/Epitope</th>
<th>( \delta \pm \text{SE} \text{ (days}^{-1}\text{)} )</th>
<th>( t_{1/2} ) (h)</th>
<th>( r^2 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flu/D(^b)NP366</td>
<td>0.88 ± 0.03</td>
<td>19.0 ± 0.96</td>
<td></td>
</tr>
<tr>
<td>Flu/D(^b)PA224</td>
<td>0.82 ± 0.09</td>
<td>20.4 ± 0.66</td>
<td></td>
</tr>
<tr>
<td>HSV/K(^a)gB498</td>
<td>0.46 ± 0.04</td>
<td>35.9 ± 0.78</td>
<td></td>
</tr>
</tbody>
</table>

* The decay rate constant (\( \delta \)) for the number of APC was estimated for the NP and PA epitopes using linear regression of the natural log-transformed data. The best-fit exponential decay constant obtained from linear regression of natural log-transformed data and SE is indicated, as well as the estimated \( t_{1/2} \) for the decay. Note that these rates do not consider any APC production after the peak, and thus estimates of the minimum decay rate.
slow and constant decay of APC indicates that the presence or absence of APC is not an on/off switch. Experimentally, the measured duration of Ag presentation will depend on the threshold for detection of APC in the assay system. For example, using in vivo proliferation assays of CFSE-labeled CD8+ T cells, whether 10^5 or 10^6 detector cells are transferred may affect the threshold of detection of Ag presentation, and thus the measured duration of Ag presentation (45). In a highly sensitive assay system (i.e., transfer of a small number of responder cells), the detection threshold may be just one APC. From our data, we would estimate the threshold of one remaining APC to occur at days 15.7, 12.7, and 15.5 postinfection for flu-NP, flu-PA, and HSV-gB, respectively. Using this criterion, the duration of presentation of flu-NP and HSV-gB would appear to be similar. In the case of flu-NP-specific APC, this is achieved through the generation of high peak APC numbers and a t_{1/2} of \( \approx \)20 h, whereas HSV-gB is characterized by low peak numbers, but a slower decay (Fig. 1). However, an assay system with a high threshold for detecting APC and requiring 100 APC to stimulate CFSE dilution would suggest that Ag presentation ended earlier, and that the duration of Ag presentation was shorter for HSV-gB than for flu-NP (HSV-gB and flu-NP crossed the threshold of 100 APC remaining on days 5.76 and 10.48, respectively), and that presentation of flu-PA ended at the same time as HSV-gB (5.67 vs 5.76 days). Thus, more rigorous kinetic analysis of Ag-presenting cell numbers is required to understand and compare Ag presentation dynamics in different infections.

Production of APC during acute infection

Following infection, the number of APC in the draining LN increases rapidly, reaching a peak at approximately day 1 in the case of HSV-gB, or day 3 in the case of flu-NP and flu-PA. Regardless of the viral infection examined, the t_{1/2} of APC after reaching their peak production was constant over the period of decline of APC (Table I). This suggests that the decay of APC is constant throughout the entire course of infection, including during the generation of APC. Using this information, the decay rate was used to calculate the rate at which Ag-loaded APC must be produced in early infection to produce the APC dynamics observed in vivo (Equation 1). We note that APC production may be composed of a number of discrete processes, including peptide loading onto pre-existing cells, migration of cells (with or without peptide preloaded) into the LN, or division of existing APC. The contribution of these different processes is unknown; therefore, we group them as APC production for simplicity.

Comparison of the rates of APC production for the three epitopes revealed substantial differences in the timing and rate of production between the epitopes. For example, production of HSV-gB-presenting cells was rapid, peaking at \( \approx \)67 cells/hour within 4–6 h of infection (Fig. 2). The peak production of the flu-PA and flu-NP APC occurs substantially later, at 48–53 and 53–60 h postinfection, respectively, and is substantially larger (\( \approx \)240 and \( \approx \)4670 cells/hour, respectively) than for HSV infection. This large difference in production rates is the key determinant of the peak APC number achieved. Because APC decay is relatively similar between the different epitopes, and slow compared with the rate of production, differences in the decay rates cannot account for the major difference in peak APC number.

The production of HSV-gB-presenting APC was not only highest immediately after infection, but was also largely completed within the first 2 days after infection. The peak number of APC and rate of APC production differs substantially between the flu-NP and flu-PA epitopes. Despite this, the overall pattern of APC production was very similar for both epitopes; APC production was negligible in the first day after infection, and most APC production occurred over a 2-day period between 1 and 3 days. This suggests that despite the significant differences in the timing of APC production, there is a shared pattern of production for the two viruses. That is, once APC production is initiated, it occurs in a burst over \( \approx \)2 days. This rapid production is followed by exponential decay of the cells produced during the burst period. The time taken to initiate the APC burst may reflect the length of time taken to reach high levels of Ag or inflammation in the different infections, but once this is achieved both HSV and flu infections follow a similar pattern of APC migration and survival (i.e., a burst of production over 2 days, followed by exponential decay). Furthermore, the timing of the peak APC production also correlates with the overall magnitude of presentation (i.e., flu-NP > flu-PA > HSV-gB APC), suggesting that if the burst in APC production is initiated early, it may lead to lower total number of APC. Further analysis would be required to determine whether such a trend held to be generally true across different viruses.

Drivers of APC and LN cell accumulation

The pattern of APC production described above is consistent with a scenario in which a resting LN is quiescent until it receives an inflammatory stimulus that initiates the rapid burst of APC production over \( \approx \)2 days. During infection, lymphoid cells are exposed to many inflammatory signals that influence the progression of the immune response. These include cytokines that may initiate cell activation or differentiation, and chemokines and adhesion molecules that may influence the recruitment of cells into the LN (25). Such inflammation may be driven by the following: 1) innate or adaptive immune responses to virus; 2) viral cytotoxic effect during viral replication in infected cells; or 3) direct mechanical injury during inoculation.

To elucidate the impact that viral dynamics might have on driving Ag presentation, we examined the viral replication and control (Fig. 3). HSV-1 infection is achieved by percutaneous injection of the footpad with \( 4 \times 10^5 \) PFU (39, 46). Twenty-four hours after
infection, 5.2 × 10^6 PFU (an 8-fold decrease in viral load) was detected at the inoculation site and virus decayed slowly over the subsequent week (39). By contrast, influenza virus infection is achieved by intranasal inoculation of 3.2 × 10^6 PFU HKX31 virus (23). After 1 day, significant viral replication occurred, increasing the lung viral load to a geometric mean value of 5.1 × 10^6 PFU (160-fold increase). This level increased further over the next 2 days, reaching a peak 3.1 × 10^7 PFU (1000-fold increase) before declining from day 5 (47).

The dynamics of HSV-1 infection are consistent with injection of an initial bolus dose of virus (or Ag) delivered in a relatively traumatic manner (by injection using a fine gauged needle). This implies a high level of Ag and inflammation would be present soon after inoculation, consistent with the early initiation of APC production and rapid activation of CD8^+ T cells following infection (32). Influenza virus infection involves the relatively atraumatic delivery of virus by intranasal infection. This is followed by rapid viral growth over the subsequent days. The method of administration is likely to induce only low-level inflammation following infection. The number of APC in influenza was not substantially increased until 24 h postinfection, peaking at about day 2–3. This suggests that virus growth, and associated cytopathology, are required before DC migration and APC production commence.

LN swelling is a cardinal sign of inflammation. Thus, the rate of accumulation of cells in the LN provides an independent measure of the extent of inflammation. To gain insight into the level of inflammation induced by the different infections, we calculated the rate of migration of cells into the LN using total LN cells, as well as the influx of APC (Fig. 3). Note that in Fig. 3 we do not factor in APC decay in calculating the rate of change in APC levels. This allowed a consistent comparison between LN and APC dynamics. Consistent with the rapid kinetics of presentation of the HSV-gB epitope, early accumulation of LN cells was observed following HSV inoculation (Fig. 4), with the peak rate of LN cell migration occurring within just 3 h after infection (Fig. 3). Compared with HSV infection, the accumulation of LN cells in influenza infection was slower, with little accumulation before 24 h and peak rate of accumulation occurring at day 2 postinfection. This is consistent with the slower kinetics of APC production in influenza infection. Despite this, the overall expansion of cells in the LN was remarkably similar in both infections between 5 and 7 days after infection (geometric mean of 1.34 × 10^7 cells vs 1.52 × 10^7 cells, respectively; p = 0.12), despite differences in the dynamics of infection and the location of the LN draining the sites of infection.

Although the rapid influx of cells into the LN and the rapid accumulation of APC were initiated at a time of high viral load, high viral loads were insufficient to sustain this influx. A decrease in both APC and LN production occurred at about day 3 in influenza infection despite persistent high viral levels until day 5 (Fig. 3). Thus, high virus levels (and presumably Ag levels) were not sufficient to maintain high levels of Ag presentation out to day 5. This probably represents the rapid accumulation and activation of APC in early infection, which then decreased despite persistent
virus. This is consistent with the transient expression of chemokines and other factors responsible for recruiting cells into the LN (48), resulting in a short-lived burst of cell migration. The continuing presence of virus and viral Ag was unable to drive further APC production in the absence of further cell recruitment. Similarly, NKT cell responses are terminated early despite the persistence of ongoing infection (49). This suggests that innate immune mechanisms may operate for a short window in early infection to drive rapid APC accumulation, but that this response is self terminating despite ongoing infection.

The influx of LN cells in both cases is likely to be driven by the same factor: inflammation. This inflammation may occur as a result of tissue damage due to trauma, innate responses to virus, adaptive immune responses to virus, or viral cytopathic effect. The difference in timing of Ag presentation observed between these two infections suggests that the tempo of these events differs between the two modes of infection. For example, the delay in initiation of LN cell migration in influenza infection implies that this requires active viral replication (which may induce inflammation either by increasing the level of virus and innate responses to virus, or by viral cytopathic effect on infected cells). In HSV infection, the extremely rapid onset on LN cell migration (and Ag presentation) is associated with initial trauma at the inoculation site, or the innate immune response to the virus in the challenge dose, triggering inflammation before significant replication occurs. Indeed, there is little evidence of significant HSV virion replication, with viral levels declining from the time of inoculation (Fig. 3). It is interesting to note that administration of HSV by flank inoculation leads to a delay in the peak of Ag presentation (50), suggesting that the infection route per se rather than the virus itself may play an important role in the timing of Ag presentation, as discussed above.

**Modeling the dynamics of Ag presentation in vivo**

The analysis above demonstrates that the production of APC is temporally coincident with the influx of LN cells during the early phase of infection. This implies that the dynamics observed in our data could be effectively described using mathematical approaches. That is, APC production should be predictable from a knowledge of LN cell number (or more specifically, its rate of change) and viral load. Simplistically, (APC production) = (LN cell influx) \(\times\) (viral load). Mathematical modeling provides a mechanism for quantitatively testing whether such mechanistic predictions are consistent with the data. By describing our predictions as mathematical formulae, we can provide a quantitative test of the concordance between experimental data and mathematical model. Using this approach to test relationships between different experimentally measured parameters can allow us to build a complex model of what forces drive APC production.

The experimental data available include level of virus (which may be equated to level of Ag), number of LN cells, and number of APC. The rate of change of LN cells (indicative of inflammation) can be calculated from the LN cell data. By examining how well different models incorporating these variables fit the available data, we can attempt to dissect the underlying mechanisms. For example, if the production of APC \((A)\) were simply dependent on the amount of viral Ag present \((V)\), measured simply as the level of virus present) and decay of APC is exponential (with rate constant \(\delta\)), we might predict that the rate of change of APC should be described as follows:

\[
\frac{dA(t)}{dt} = kV(t) - \delta A(t) \tag{4}
\]

Alternatively, because presentation requires viral Ags to be presented on a subset of LN cells, the rate of production of APC may be a function of the level of Ag and the number of LN cells \((L)\) that can present Ag, as follows:

\[
\frac{dA(t)}{dt} = kV(t)L(t) - \delta A(t) \tag{5}
\]

Notably, both of the above models (Equations 4 and 5) give a peak in Ag presentation that is significantly later than that observed in vivo and leads to large sum of square errors. This is a result of the production rate of APC in these equations being solely dependent on the viral load or the viral load and the number of cells in the LN \((L)\). Both viral load and LN cell numbers remain high out to day 5 postinfection, whereas APC levels have already begun declining and production has ended (Fig. 2); equations 4 and 5 therefore cannot account for the earlier peak in APC levels. This suggests that more complex models of APC dynamics may be required.
Inflammation occurs as a consequence of tissue damage, innate immunity to virus, or the adaptive immune response to pathogen infection. It is therefore difficult to find a single measure of inflammation other than by observing its effects on cell behavior. In addition to influencing the behavior of lymphocytes, inflammation provides a potent stimulus for both the maturation and migration of DC into the LN. It also promotes increased vascular permeability and lymph flow into the LN (51). However, inflammation and DC immigration in the absence of Ag will not lead to Ag presentation. Although we have no direct measure of overall inflammation, the rate of influx of LN cells (ϕ) would provide an approximation of the magnitude of the inflammatory stimulus. Therefore, a model incorporating both the magnitude of LN cell influx, rather than simply LN cell levels, and the level of viral Ag may predict the accumulation of APC in vivo. The simplest model incorporating these two critical features driving inflammation can be described by the following:

\[ \frac{dA(t)}{dt} = k_{LV}V(t)\phi(t) - \delta A(t) \]  

Using this approach, we can obtain estimates of two parameters: \( k_{LV} \), the APC production coefficient, and \( \delta \), the loss rate constant of APC.

Fitting this model (Equation 6) to the experimental data provided good fits for the flu-NP epitope, but was less robust for the other two epitopes (see Fig. 5a). As might be expected, the \( t_{1/2} \) calculated by this method (taking into account potential APC production) is shorter than the observed \( t_{1/2} \) that we estimated earlier (using linear regression analysis), which reflected the balance of APC production and decay during this period (Table II). However, using this approach, production of APC is predicted to terminate before day 2 and 5 postinfection, and thus, production should have a minimal effect on the decay rate of APC. This is borne out because the \( t_{1/2} \) estimated by the two methods are usually very similar. Indeed, in some cases, the \( t_{1/2} \) estimated from Equation 6 is longer than that estimated from linear regression, largely because of the failure of the model to produce a high enough peak in APC numbers.

The model fit to the experimental data on the number of APC presenting the HSV-gB and the flu-PA epitope is relatively poor, predicting the peak level of presentation to occur much earlier or later than the observed peak in the data. This also led to a calculated mean \( t_{1/2} \) (Table II) that was unexpectedly longer than that calculated from linear regression analysis in which production is not considered. As indicated above, this is a limitation of the fitting procedure used, in which less emphasis is placed on fitting peak APC data to better fit low APC data at later time points. Thus, although the production of APC is coincident with the influx of LN cells, it cannot be predicted by a simple function of viral load and LN cell flux.

Table II. Estimated APC production and decay constants using different modelsa

<table>
<thead>
<tr>
<th>Virus/Epitope</th>
<th>Mean ( k_{LV} ) (( 10^{-10} ) APC · PFU(^{-1}) · LNC(^{-1}))</th>
<th>Mean ( \delta ) (day(^{-1}))</th>
<th>( t_{1/2} ) (h)</th>
<th>Sum of square error</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flu/DNP(_{666})</td>
<td>5.06 ± 0.99</td>
<td>0.99 ± 0.10</td>
<td>16.8</td>
<td>69.7</td>
</tr>
<tr>
<td>Flu/DP(_{224})</td>
<td>0.28 ± 0.09</td>
<td>1.10 ± 0.15</td>
<td>15.1</td>
<td>249.2</td>
</tr>
<tr>
<td>HSV/K(_{68})</td>
<td>1.97 ± 0.51</td>
<td>0.18 ± 0.05</td>
<td>92.3</td>
<td>178.2</td>
</tr>
</tbody>
</table>

a The APC production coefficient in terms of LN cell (LNC) or LDC is represented by \( k_{LV} \), and the level of viral Ag may predict the accumulation of APC in vivo. The simplest model incorporating both the magnitude of LN cell influx, rather than simply LN cell levels, and the level of viral Ag may predict the accumulation of APC in vivo. The simplest model incorporating these two critical features driving inflammation can be described by the following:

\[ \frac{dA(t)}{dt} = k_{LV}V(t)\phi(t) - \delta A(t) \]  

Using this approach, we can obtain estimates of two parameters: \( k_{LV} \), the APC production coefficient, and \( \delta \), the loss rate constant of APC.

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Understanding the role of migrating LDC

The model described by Equation 6 above uses the total LN cell number as a surrogate measure of the level of inflammation. This is assumed to reflect the level of migration and maturation of DC. However, DC are a phenotypically and functionally heterogeneous population of cells. Ag presentation during viral infection appears to be largely conducted by DC that reside permanently in the LN (LN-resident DC), known as CD8\(^{+}\) DC, whereas the majority of lymphoid cells entering (or proliferating within) the LN are not DC. Therefore, we used direct labeling of DC in the lung to evaluate the contribution LDC (a type of tissue-derived DC) might make to the LN DC population. Tissue-derived DC (or interstitial DC, CD8\(^{α}\)-CD11b\(^{+}\) DC) play an important role in carrying Ag to the LN. Within the LN, Ag is transferred to LN-resident DC for presentation to T cells, a phenomenon known as cross-presentation (16, 52). The migration of LDC from the lung to the LN during the first few days of influenza virus infection has been measured by enumerating the number of LDC that incorporated the 5,6-diacetylcarboxyfluorescein administered intranasally before infection (23).

The peak number of LDC (≈2400 cells) accumulated 24 h after infection. Comparing the kinetics of LDC with that of the number of APC in the lung, the following are clear: 1) the peak number of LDC occurs significantly earlier than the peak in APC (36 h in the case of flu-NP, and 24 h earlier in the case of flu-PA), and 2) the peak number of APC can be many fold larger than the peak number of LDC (22- and 1.6-fold for flu-NP and flu-PA, respectively). Although it is possible that the apparent amplification from LDC to APC arose due to incomplete labeling of LDC, it is also plausible that this difference reflects the amplification process predicted to be an essential component of cross-presentation, involving the transfer of viral fragments or Ag from one APC to another (53, 54).

When Ag transfer occurs between LDC (D) and LN-resident DC, the change in APC over time may be reflected by a simple function of the LDC number together with the level of Ag present, as follows:

\[ \frac{dA(t)}{dt} = k_{DV}V(t)D(t) - \delta A(t) \]  

Using this function to model APC number, we find that this provides a good fit for the experimental data for PA-specific APC numbers and a reasonable fit for NP-specific APC (Fig. 5b). The model fit for the NP epitope peaks earlier and lower than the data, which leads to a longer \( t_{1/2} \) for NP-specific APC (Table II) compared with the linear regression analysis. This is again due to the limitation of the fitting procedure, but nevertheless suggests that cross-presentation of Ag from lung DC is not incompatible with the data.
Discussion

The CD8+ T cell response is essential for the early control of many viral and other pathogen infections. A delay in CD8+ T cell activation is thought to be a major underlying reason for the inability of CD8+ T cell-mediated immunity to control HIV infection (33, 55). Although there has been extensive analysis of the dynamics of the CD8+ T cell response in mice and humans, there has been only limited analysis of the dynamics of the priming stimulus, that is, the level of Ag presentation driving the activation of CD8+ T cells. In an effort to understand how Ag presentation shapes the T cell response, we have used an in vitro culture system data to track the number of APC in the draining LN of mice infected with influenza virus and HSV and to study their kinetics during infection.

The experiments showed that the decay of APC following primary infection was exponential, with a $t_{1/2}$ of 19.0, 20.4, and 35.9 h for the flu-NP, flu-PA (influenza infection), and HSV-gB (HSV infection) epitopes, respectively. Several potential mechanisms could lead to this type of decay in vivo. These include the following: 1) APC death due to CTL killing; 2) natural APC turnover due to division, natural death (apoptosis), or migration of cells from the lymphoid tissue; 3) infection-driven APC turnover, death, or migration; and 4) decay of peptide-MHC (p-MHC) complexes on the surface of cells. The decay kinetics of APC elucidated from our study provides some insight into the contribution that these different factors might have to the overall decay of Ag presentation following primary infection. For example, if APC removal were due to CTL killing (27), then it might be anticipated that the number of APC would be proportional to CTL number. However, the $t_{1/2}$ of cells presenting the NP epitome remains remarkably constant from day 3–15, despite large fluctuations in number of CTL during this time (56, 57). Moreover, as we have demonstrated previously, this decay is unaffected in perforin-deficient animals in primary infection, despite clear evidence for killing of APC in secondary infection (23, 25, 27). In secondary infection, the growth in APC number appears very similar to that seen in primary infection during the first days of infection, but then precipitously decreases at about day 2. In the case of secondary infection, it appears that CTL killing of APC plays a significant role in the decay of Ag presentation.

A second mechanism regulating APC kinetics is that the observed decay of APC may be attributable to the natural death or migration rate of DC. A small number of studies have investigated the natural turnover rate of DC in vivo in different organs using BrdU labeling (5, 6). These studies measured the rate of incorporation of BrdU by newly produced cells and calculated that DC divide every 1.5–3 days at steady state. By inference, the natural death rate of DC must be similar (because the overall number of DC is stable). Both antigenic and strong inflammatory stimuli were found to increase the turnover of DC (5). In that study, it was unclear that the death rate was also increased, because the number of DC may no longer be in equilibrium. However, if natural turnover of DC were the major cause of APC decay, one would expect that the decay rate should be fixed for all epitopes and in different infections. The significantly slower decay of APC in HSV-1 infection suggests that some intrinsic natural turnover rate of DC is unlikely to explain the dynamics of APC decay in vivo.

Despite this, the slower loss of APC in HSV-1 infection does not exclude the possibility that decay is due to DC turnover; the differing rates observed in HSV and influenza infection may simply reflect infection-specific factors. A number of viral pathogens have been shown to modulate Ag presentation during infection. For example, lymphocytic choriomeningitis virus (clone 13) can induce immunosuppression through CD8+ T cell-dependent death of DC (and thus may induce both viral and CTL killing of cells) (58). Murine CMV (59) and measles virus (60) can both inhibit DC function, and the latter also promotes DC apoptosis (61). Such effects would significantly impact on DC turnover. Therefore, it is conceivable that acute influenza virus infection may stimulate faster DC turnover or recruit a different subset of DC (either due to its anatomical location in the lung, recruitment of different DC, or different activation of DC cells via innate immune recognition) (16, 46). These changes in DC phenotype or activation state may directly contribute to the observed differences in APC decay. Further experimental data would be necessary to support this interpretation.

In addition to factors that influence the life and death of APC themselves, the role of p-MHC complexes on the APC surface must be considered. The number and stability of these p-MHC complexes are thought to play a central role in fostering T cell interactions that influence both the magnitude and immunodominance hierarchy of CD8 T cell responses to infection (62–64). Previous studies have analyzed the stability of p-MHC class I (p-MHC-I) complexes formed during Ag loading of TAP2-deficient cell lines, such as RMA-S. Such studies have demonstrated that the $t_{1/2}$ of p-MHC complexes at 37°C is ~1–6 h (65, 66). In these studies, the observed $t_{1/2}$ of p-MHC-I on the surface of cells may be affected by both the biochemical degradation and the rate of internalization of surface p-MHC-I by the cell (67). Recent in vitro studies suggest that the $t_{1/2}$ of p-MHC-I on the surface of mature DC is significantly longer than on immature cells, and that arrest of cellular metabolic activity increases the $t_{1/2}$ of p-MHC-I on the surface (67). Thus, it seems likely that in resting cells the surface $t_{1/2}$ of MHC class I is largely determined by the rate of cellular degradation, whereas in mature DC this $t_{1/2}$ is significantly extended due to decreased degradation and increased persistence on the cell surface. This model is consistent with the recent observation that prolonged survival of MHC class II on the surface of mature DC is mediated by reduced ubiquitination (68, 69). Additional studies on $t_{1/2}$ will extend our understanding of how the $t_{1/2}$ of p-MHC-I affects the duration of Ag presentation in vivo.

The timing of initial APC production may play a crucial role in mediating early viral control. The present study shows that differences in the timing and magnitude of APC production can occur in different infections, highlighting the role of pathogen-specific factors in determining APC kinetics. In particular, the very rapid initiation of Ag presentation in experimental HSV infection suggests that the route and magnitude of challenge, and not extensive viral replication, are important factors. By contrast, natural infection probably involves a small initial inoculum, followed by extensive viral proliferation to generate sufficient antigenic and inflammatory stimuli to promote LN cell migration and Ag presentation. Influenza virus infection appears to closely fit into this schema, with a 24-h delay before the initiation of Ag presentation.

The production of APC requires both Ag and inflammation. Our study suggests that the production of APC is more closely correlated to the level of inflammation and LN cell influx than it is to Ag levels, because APC production in influenza infection has largely ceased by day 4 (like LN cell influx), despite continued high viral levels until day 5 (Fig. 3). It is interesting to note that in both HSV and flu infection, APC production occurs over a ~2–day period, despite starting later in flu. This suggests that once APC production is initiated, it occurs in a stereotyped burst that takes 2 days, but then largely ceases. The failure to produce more APC-presenting influenza epitopes on days 4 and 5 may arise due to exhaustion of the supply of LDC despite the persistence of high levels of virus, as has been suggested previously (41). This is consistent
with a refractory period after inflammatory stimuli, during which further Ag presentation is arrested (14, 41).

Interestingly, both the production of APC and the influx of LN cells cease at day 3–4 after influenza infection. Why LN cell influx is also down-modulated at this time despite high levels of virus in the lung until at least day 5–6 is unclear. A simplistic interpretation might be that the LN is full. It is also possible that the early inflammatory response to virus is self-limiting, despite the persistence of virus, perhaps due to some form of feedback regulation of the inflammatory response. Interestingly, this corresponds to local changes in chemokine production, which may play a role in modulating the LN influx at this time (day 3–5) (48). Whatever the cause, the temporal association between the production of APC and the growth in total LN cell numbers suggests that the two may be governed by similar factors. If early inflammation and APC production truly follow such a stereotyped pattern, it would imply a limited benefit to more potent adjuvants in vaccination, once a maximal level of LN cell influx is achieved.

Acknowledgments
We thank Frank Carbone for providing experimental data on HSV-1 infection.

Disclosures
The authors have no financial conflict of interest.

References

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