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Cutting Edge: Prolonged Antigen Presentation after Herpes Simplex Virus-1 Skin Infection

Angus T. Stock, * Scott N. Mueller, ** Allison L. van Lint, *** William R. Heath, † and Francis R. Carbone† *

It has been reported that MHC class I-restricted Ag presentation persists for only a short period following infection with certain pathogens, declining in parallel with the emergence of specific CTL activity. We have examined this issue in the case of murine infection with HSV-1. We found that the period of Ag presentation capable of priming naive CD8+ T cells is comparatively prolonged, persisting for at least 7 days after infection, and continuing despite the appearance of localized CTL activity. Ag presentation was abbreviated to 3 or 4 days postinfection by surgical excision of the inoculation site early after infection. This intervention attenuated the size of the primary CTL response, implying that prolonged presentation is necessary to drive maximal CTL expansion. Combined, these data show that, in some types of infection, CTL priming can extend well beyond the first 24–48 h after primary inoculation. The Journal of Immunology, 2004, 173: 2241–2244.

Cellular immunity is initiated by the acquisition and presentation of foreign Ags by professional APC. Although it has been shown that these events commence within hours of infection (1–4), the duration of Ag presentation remains less well defined. Initial in vitro studies suggested that naive CD8+ T cells require only brief antigenic stimulation, in some cases as little as 2 h, to enter an autonomous program of division and differentiation (5). At face value, this appears consistent with recent in vivo studies that have shown surprisingly transient levels of Ag presentation after infection with certain viruses, bacteria, and parasites (1, 3, 6). In these latter studies, Ag presentation could be detected within hours of infection but appeared to be extinguished variously by a day or two after its initiation. In the case of Listeria monocytogenes infection, the loss of Ag presentation coincided with the emergence of lytic Ag-specific CTL in the lymphoid organ, giving rise to the notion that the period of Ag presentation was controlled by a feedback mechanism involving the lysis of the APCs by the expanding CTL population (6). Given these demonstrations of surprisingly brief periods of presentation, we have examined the kinetics of Ag presentation following skin infection with HSV-1. In this study, we show that Ag presentation capable of priming resting CD8+ T cells continues in the face of strong CTL activity, persisting far longer than articulated in recent studies, and that this prolonged presentation is necessary for maximal virus-specific T cell expansion.

Materials and Methods

Mice, virus, and peptides

C57BL/6, gBt-I, gBt-1.L × B6.SJL-Ppuc*Pep3/PBoyl (gBt-I × B6.Ly5.1), and OT-I mice were obtained from Department of Microbiology and Immunology (University of Melbourne). The gBt-I and OT-I TCR-transgenic mice are specific for the H-2Kb-restricted immunodominant HSV-1 epitope gB198–205-SLIEFARL (7) and OVA-derived epitope OVA257–264-SIINFEKL (8), respectively. The KOS strain of HSV-1 was propagated and titered on Vero cells in MEM (MEM with 10% heat-inactivated FCS, 23.83 g/L HEPES, 4 mML-glutamine, 50 μg/mL streptomycin, 100 U/mL penicillin, 100 μg/mL gentamicin, and antibiotics).

Virus infections and inoculation site excision

C57BL/6 mice were inoculated either with 4 × 105 PFU of HSV-1 injected s.c. into each hind footpad or with 1 × 106 PFU of HSV-1 after flank scarification. Inoculation by flank scarification is described in detail elsewhere (9, 10). At specified times after HSV-1 flank infection, the inoculation site or the corresponding area on the contralateral flank was excised from anesthetized mice by surgical excision of the inoculation site early after infection. The Journal of Immunology, 2004, 173: 2241–2244.

Determination of viral titer in the dorsal root ganglion (DRG) after HSV-1 infection

C57BL/6 mice were infected with HSV-1 via flank scarification and sacrificed after 3 days, and the DRGs innervating the infected dermatome (thoracic DRG levels 7–13) were removed, pooled, and frozen at −70°C in MEM. Samples were thawed at 37°C and homogenized, and the amount of infectious virus in each sample was determined using a standard PFU assay on confluent Vero cell monolayers.

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3 Abbreviations used in this paper: DRG, dorsal root ganglion; gB, glycoprotein B; DC, dendritic cell.
**APC detection assay**

The lacZ-inducible glycoprotein B (gB)-specific T cell hybridoma HSV-2.3E2 was used for the detection of gB epitope-bearing APCs in individual popliteal lymph nodes after HSV-1 footpad infection as previously described (2). Briefly, 2-fold serial dilutions of collagenase-digested lymph node cells, starting at $10^6$ cells/well, were cultured in 96-well plates with $10^5$ hybridoma cells for overnight culture before performing 5-bromo-4-chloro-3-indolyl β-D-galactoside assays.

**In vivo CTL and proliferation assay**

Four-hour in vivo CTL assays were performed as previously described (11). For proliferation assays, lymph nodes from gBT-I or OT-I donor mice were harvested, made into single-cell suspensions, and labeled with CFSE (2.5 µM) for 10 min at 37°C. Cells were washed, and $1 \times 10^6$ CFSE-labeled lymphocytes were adoptively transferred via i.v. tail vein injections at various times after HSV-1 infection. Sixty hours after transfer, the draining lymph node(s) were harvested and stained with anti-CD8-allophycocyanin. Stained cell suspensions were analyzed by flow cytometry, collecting between $5 \times 10^2$ and $1 \times 10^4$ CFSE–CD8^+ live lymphocytes.

**Flow-cytometric analysis of gBT-I expansion**

C57BL/6 mice that received $1 \times 10^3$ gBT-I × B6.Ly5.1 lymph node cells 1 day earlier were inoculated with HSV-1 via flank scarification. Seven days after infection, mice were killed, and their spleens were removed. Single-cell suspensions were stained with anti-CD8-FITC, anti-CD45.1-biotin, and streptavidin-allophycocyanin. Stained cell solutions were analyzed by flow cytometry, collecting between $5 \times 10^2$ and $1 \times 10^4$ CFSE–CD8^+ live lymphocytes.

**Results**

**Persisting Ag presentation after HSV-1 infection**

We had previously examined Ag presentation in the draining lymph node after HSV-1 infection by using a T cell hybridoma specific for the HSV-1 immunodominant determinant from gB to show that presentation began between 4 and 6 h after footpad infection (2). Using this approach to measure the longevity of Ag presentation after HSV-1 infection, we found that, although reduced from maximal day 2 levels, gB presentation persisted within the popliteal lymph nodes at detectable levels until ~8 days after footpad infection (Fig. 1A). To determine whether this persisting presentation was capable of priming gB-specific CTL precursors, we transferred T cells derived from a gB-specific TCR-transgenic mouse (gBT-I) (7) at progressively later times after footpad infection. These T cells were labeled with CFSE before transfer, and the dilution of fluorescence intensity signifying proliferation was assessed by flow cytometry 60 h after transfer. Fig. 1A shows that strong proliferation could be detected in the draining lymph node when T cells were transferred as late as 5 days after footpad infection, and some level of presentation appeared to persist until at least day 7 after footpad infection.

**Early surgical removal of the site of skin inoculation limits the spread of lytic virus infection**

In an effort to limit the extent of infection, we used a modified flank scarification model of HSV infection. Infection with HSV results in the initial replication in the epithelium immediately adjacent to the site of inoculation before the movement of the virus into the thoracic DRGs and the ultimate re-emergence of virus along the band of skin innervated by the sensory ganglia to give a band of zosteriform lesions. Surgical excision of the primary inoculation site at progressively earlier times after infection appeared to stop the spread of virus, with lesions failing to develop if surgery was performed before 24–36 h after inoculation (Fig. 2A). Consistent with this, recovery of lytic virus from the DRGs was reduced by >1000-fold if surgery was delayed 24 h after infection, and no replicating virus was detected when skin infection was limited to the first 8 h after inoculation (Fig. 2B). These results argue that the earlier excision of the site of flank infection limits lytic replication to the site of inoculation and the period of time between inoculation and surgical intervention.

**Ag presentation continues for days after the cessation of lytic infection, even in the presence of CTL effector activity**

Given the preceding results, we used this combination of flank infection followed by skin excision to examine how long presentation persisted after cessation of HSV replication. Persistence of in vivo presentation was again measured using the transfer of CFSE-labeled gBT-I cells at various times after infection. Fig. 3A shows that, in mice whose inoculation site was excised 8 h postinfection, CD8^+ gBT-I T cells proliferated strongly in the draining brachial lymph node when transferred 2 days after infection. gBT-I T cells continued to divide, albeit at reduced levels, when transferred 4 days after 8-h infection, whereas T cell proliferation was only marginally above background levels when transferred 7 days after infection in 8-h excision mice. If surgical excision was conducted 24 h postinoculation, gBT-I T cells proliferated strongly in the day 4 transfer mice, and low-level donor cell proliferation was observed when transferred 7 days after infection. Thus, in manipulated mice, Ag presentation capable of driving strong proliferation of donor gBT-I T cells continued in the draining lymph node for another...
of gB-T-I CD8$^+$ T cells were adoptively transferred into infected recipients. Sixty hours posttransfer, mice were sacrificed, and the division of donor CD8$^+$ gBT-I T cells in the draining brachial lymph node was determined by flow cytometry for the dilution of their CFSE fluorescence. Histograms are gated upon live CD8$^+$ CFSE$^+$ lymphocytes, and a representative plot is shown of five to seven mice per time point. Inset values represent the mean and SD for the percentage of gBT-I cells that entered division. Mean background cell division in the absence of infection was 6% with 4% SD. $b$, The level of anti-gB cytolytic activity present in the draining brachial lymph node was measured 4 days after flank infection using a 4-h in vivo CTL assay. Shown is the average of six to eight mice per group, with error bars representing SD.

Discussion

We have found Ag presentation capable of driving resting CD8$^+$ T cells into cycle persists in the draining lymph node for at least 7 days after skin infection with HSV-1. Although a similar period of prolonged presentation has been reported following Sendai virus infection (12), a number of more recent studies have found the corresponding period to be far more abbreviated (1, 3, 6). Although our use of relatively large numbers of transgenic T cells may have accentuated the effect of residual presentation, an identical approach showed presentation to last only 3 days after L. monocytogenes infection despite ongoing bacterial replication (6), and even shorter periods of presentation were reported after infection with malaria and vaccinia virus (1, 3). In contrast, our findings fit with a model where the duration of Ag presentation is prolonged, persisting beyond the life of the primary lytic infection. Indeed, we show that a cutaneous HSV-1 infection that lasted for only 8 h, maintained some level of class I-restricted Ag presentation for another 4 days within the draining brachial lymph nodes.
FIGURE 4. An infection period of >24 h is required to prime maximal CTL response. A total of 1 × 10⁶ gBT-I × B6.Ly5.1 lymph node cells was adoptively transferred into congenic C57BL/6 recipients 1 day before HSV-1 infection via the flank scarification method of inoculation. a, At 2, 8, or 24 h postinfection, the inoculation site was surgically excised or left intact (w/o). b, At 2 h postinfection, the corresponding area of skin to the inoculation site on the contralateral flank was excised (excision) or left intact (w/o). Seven days after infection, the proportion of splenic CD8⁺ T cells of gBT-I origin was assessed by flow cytometry on the basis of Ly5.1 and CD8 expression. Shown is the percentage of splenic CD8⁺ T cells that are of gBT-I origin, with averages from 6 to 16 mice per time point and error bars representing SD. Statistical significance between truncated (2, 8, or 24 h) and wild-type infections (w/o) was assessed using Student’s t-test, and values are shown: *, p < 0.05; **, p < 0.01; or ***, p < 0.001.

Following L. monocytogenes infection, the cessation of class I-restricted presentation coincided with the acquisition of effector capabilities by the Ag-specific CTL pool (6), leading to the suggestion that presentation is transient due to the CTL-mediated clearance of APC. In this study, we see presentation persisting well beyond the peak of CTL activity, arguing that class I-restricted Ag presentation is not extinguished by armed effector T cells in this infection. This is not to say that CTL can play no role in reducing the level of presentation, and indeed, CTL feedback may explain the drop from its observed day 2 maximum following footpad infection. Nonetheless, presentation can persist in the face of quite robust CTL activity, and to a level that appears to have some effect on the final size of the immune response.

At this point, we are unable to explain why different infections result in either prolonged or abbreviated presentation but believe that it may reflect a fundamental difference in mechanisms of CTL priming. One possibility might be a differential role of CD4⁺ T cells in the respective responses, because these T cells can protect dendritic cells (DCs) from CTL-mediated killing (13). Alternatively, prolongation of presentation in the case of HSV-1 infection may reflect sequestration of Ag in a manner not directly accessible to CTL. In this respect, HSV-1-specific CTL priming appears to exclusively involve class I-restricted presentation by lymph node-resident CD8⁺ DCs, suggesting that migrating skin DCs may primarily act as nonpresenting Ag carriers (9, 14). This nonpresenting state makes these skin DCs insensitive to CTL elimination, which combined with their prolonged survival (15), could mean that they act as a relatively long-lived reservoir that maintains presentation for some time after the cessation of lytic HSV-1 infection.

Unlike other infectious models (3, 16), we have found that an extended period of presentation is required for the optimal level of CTL expansion. This is perhaps not surprising in the context of a localized viral infection, where T cell activation occurs exclusively within those lymph nodes draining the site of infection. Given this anatomical restriction, presentation beyond the first day or two after infection may be required to ensure the complete scanning of the entire T cell repertoire needed to recruit the maximal number of naive CTL precursors from the circulation. In contrast, a more disseminated infection, for example, L. monocytogenes and malaria, might have ready access to a large T cell pool, requiring a shorter period of presentation for optimal T cell priming. In addition, given that T cells are retained within the draining lymph node over the course of many divisions (11, 17), it remains possible that persisting presentation during this period results in extended or repeated T cell stimulation necessary for optimal cell survival, expansion, or acquisition of full effector function (18, 19).

In summary, we have found that Ag presentation is relatively prolonged following HSV-1 infection, even in the absence of continuing replication and in the face of local Ag-specific CTL activity. Prematurely aborting Ag presentation was found to attenuate the size of the ensuing CTL response, implying that persisting presentation has some immunological relevance, at least in terms of generating maximal CTL expansion.

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