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Diminished secondary CTL response in draining lymph nodes on cutaneous challenge with herpes simplex virus

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We have shown that C57BL/6-derived CD8$^+$ CTL specific for an immunodominant herpes simplex virus type 1 (HSV-1) glycoprotein B (gB) determinant express a highly conserved Vβ10/junctional sequence combination. This extreme T cell receptor β-chain bias can be used to track the activation of gB-specific CTL in lymph nodes draining the site of HSV-1 infection. In this report we have examined the accumulation of gB-specific CTL in the primary and secondary or recall CTL responses to HSV-1 infection. We found that gB-specific cytolytic activity present within popliteal lymph nodes draining HSV-infected foot-pads peaked at day 5 post-infection during the primary response. As found previously, this correlates with the accumulation of Vβ10$^+$CD8$^+$ CTL in the activated T cell subset. Lymph node-derived cytotoxicity peaked between days 3 and 4 on secondary challenge with virus and, somewhat surprisingly, was considerably below that seen in the primary response. This reduced gB-specific cytolytic activity mirrored a near absence of Vβ10$^+$CD8$^+$ T cell enrichment found within the draining lymph nodes during this recall response, consistent with the overall diminution of gB-specific CTL accumulation in this site. Finally, there was a second wave of biased accumulation of Vβ10$^+$CD8$^+$ activated T cells within the popliteal lymph nodes well after the resolution of infection in both the primary and secondary responses. These results are discussed in terms of preferential activation of virus-specific memory T cells directly in infected tissues during a secondary CTL response at the expense of draining lymphoid organs.

Introduction

Immunological memory to virus is characterized by an enhanced clearance on challenge with the same infectious agent. Antigen-specific CTL are known to contribute to this rapid response and can independently protect individuals from subsequent infections with certain viruses (Ahmed, 1992; Doherty *et al.*, 1996). Two mechanisms have been proposed for the enhanced CTL response upon rechallenge with virus. The first centres on the increased precursor cell numbers that remain after initial antigenic stimulation (Hou *et al.*, 1994; Lau *et al.*, 1994; Moskophidis *et al.*, 1987; Ryser & MacDonald, 1979). Activation of naive T cells results in a massive expansion of specific CTL precursors so that they dominate the activated T cell subset (Butz & Bevan, 1998; Cose *et al.*, 1997; Murali-Krishna *et al.*, 1998). While many of these cells go on to die by activation-induced cell death (Razvi & Welsh, 1995), a fraction, numerically greater than the starting naive subset, survive (Busch *et al.*, 1998; Butz & Bevan, 1998; Flynn *et al.*, 1998; Murali-Krishna *et al.*, 1998). This increased precursor cell number, or burst size, directly contributes to the enhanced responses seen on subsequent encounter with antigen (Hou *et al.*, 1994). In addition, memory T cells are phenotypically different from their naive counterparts (Akbar *et al.*, 1988; Budd *et al.*, 1987). They mount a more vigorous response on subsequent encounter with specific

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antigen (Byrne *et al.*, 1988; Sanders *et al.*, 1988, 1989) and have a less stringent co-stimulator requirement (Croft *et al.*, 1994; Luqman & Bottomly, 1992; McKnight *et al.*, 1994).

Many of the phenotypic differences between naive and memory CTL reside in differential expression of adhesion molecules (Budd *et al.*, 1987; Hamann *et al.*, 1988; Jung *et al.*, 1988; Lerner *et al.*, 1989), which suggests that differential CTL migration, as well as quantitative differences in the strength of the response, could also contribute to the enhanced virus clearance evident on subsequent reinfection. For example, activated T cells rapidly downregulate CD62L (Jung *et al.*, 1988), limiting their ability to enter lymph nodes, while upregulating adhesion molecules that facilitate entry into peripheral compartments (Butcher & Picker, 1996; Mackay, 1991). A large proportion of memory CTL retain this CD62Llo phenotype, at least for some time after infection (Doherty *et al.*, 1996; Tripp *et al.*, 1995; Zimmerman *et al.*, 1996), suggesting that these cells would be activated either within the spleen or, more likely, directly within tissues harbouring replicating virus during reinfection. In either case, the loss of CD62L expression renders these memory CTL incapable of transit through the high endothelial venules and thus may limit their direct access to draining lymph nodes on subsequent infection (Butcher & Picker, 1996; Mackay, 1991).

In an attempt to understand the mechanisms involved in lymph node T cell activation upon reinfection, we have examined the primary and secondary CTL response to foot-pad infection with herpes simplex virus type 1 (HSV-1). A variety of studies have shown that CTL play an important role in eliminating this virus from the primary site of infection (Bonneau & Jennings, 1989; Nash *et al.*, 1987; Simmons & Tscharke, 1992). HSV-1-specific CTL from C57BL/6 mice recognize a K^b-restricted immunodominant determinant from glycoprotein B (gB) (Bonneau *et al.*, 1993; Hanke *et al.*, 1991; Witmer *et al.*, 1990). We have previously shown that T cells express a highly restricted pattern of T cell receptor (TCR) Vβ and junctional sequence conservation, which can be exploited to track the accumulation of gB-specific CTL in lymph nodes draining the site of infection (Cose *et al.*, 1995, 1997). Here we use the dominant Vβ expression to compare the gB-specific CTL accumulation in this site following primary infection and secondary challenge with HSV-1.

Methods

■ **Mice.** C57BL/6 mice were purchased from the Central Animal Facility at Monash University, Clayton, Victoria, Australia.

■ **Virus and cell lines.** The KOS strain of HSV-1 was propagated on Vero cells. The gB peptide with the sequence SSIEFARL (single letter amino acid code) corresponding to residues 498 to 505 in HSV-1 gB was synthesized using an Applied Biosystems model 431A synthesizer (ABI) and kindly provided by J. Fecondo, Swinburne University of Technology, Hawthorn, Victoria, Australia. The H-2^b thymoma cell line EL4 was grown in complete DMEM supplemented with 10% FCS.

■ **Injection with HSV-1 or gB peptide.** C57BL/6 mice were injected in each hind foot-pad with 4×10^5 p.f.u. HSV-1 or with approximately 5 μg gB peptide emulsified either in TiterMax (CytRx Corporation) or complete Freund's adjuvant (CFA; Sigma). After 6 weeks these mice and a group of naive C57BL/6 mice were infected in each hind foot-pad with 4×10^5 p.f.u. HSV-1 and the draining popliteal lymph nodes and the non-draining mesenteric lymph nodes were removed between 1 and 10 days later. Viable cell counts were determined for all lymph node samples by trypan blue exclusion prior to analysis. Lymph node cells were analysed directly and after 3 days in culture in wells of a 96-well flat-bottom plate at a density of 1×10^6 cells per well in 250 μl complete RPMI containing 10% FCS without exogenous antigen.

■ **Assessment of gB-specific CTL activity.** CTL lysis was assessed by a 4 h chromium release assay using ^{51}Cr (150 μCi)-labelled EL4 cells in the presence or absence of 1 μg/ml gB peptide. Varying effector-to-target ratios were used for cells analysed directly *ex vivo* with the results expressed as a percentage of specific lysis. For cells analysed after 3 days of culture without exogenous antigen, the assay was performed using serial dilutions of effectors from one well of culture. Lytic units were determined for 20% specific release and related back to the *ex vivo* number of cells in each lymph node. Thus, the results are expressed as the number of arbitrary units per lymph node able to specifically lyse 20% of the target cells.

■ **Flow cytometry.** Lymph node cells analysed directly *ex vivo* or after 3 days culture without exogenous antigen were double-stained with FITC-labelled anti-CD8 (CT-CD8a, Caltag Laboratories) and biotin-labelled anti-Vβ10 (B21.5, Pharmingen) followed by phycoerythrin–streptavidin (Southern Biotechnology Associates). Dead cells were excluded using propidium iodide and the cells were visualized on a Becton Dickinson FACScan. The lymph node cells were separated in small and large (blast) subsets based on forward- and side-scatter parameters for all analyses. Previous analysis using CD44 and CD62L markers showed that the majority of cells falling into the blast cell gate are activated (Cose *et al.*, 1997). The proportion of $CD8^+$ blasts expressing the Vβ10 element was determined by flow cytometry as the fraction of $CD8^+$ T cell blasts expressing Vβ10 receptors and is given as a percentage.

Alternatively, lymph node cells were triple-stained with allophycocyanin-labelled anti-CD8 (53-6.7, Pharmingen), phycoerythrin-labelled anti-CD25 (PC61, Pharmingen) and biotin-labelled anti-Vβ10 (B21.5, Pharmingen) followed by streptavidin–FITC (Molecular Probes). Dead cells were excluded using propidium iodide and the cells were visualized on a Becton Dickinson FACScalibur. The proportion of $CD8^+CD25^+$ cells expressing the Vβ10 element was determined by flow cytometry as the fraction of $CD8^+CD25^+$ cells expressing Vβ10 receptors and is given as a percentage.

■ **Determining virus titre.** Hind feet of mice infected with HSV-1 were removed at the ankle and individual feet were ground in a 5 ml homogenizer (Laboratory Supply, Australia) to make a 20% (w/v) suspension in RPMI supplemented with 10% FCS. These suspensions were frozen at −70 °C, then thawed rapidly and centrifuged at 12000 ***g*** for 10 min at 4 °C. The supernatant fluid was used immediately in a p.f.u. assay on Vero cells to determine the virus titre. Briefly, serial tenfold dilutions of the supernatant fluids were made in serum-free MEM and added to confluent monolayers of Vero cells in 6-well multiwell dishes (0·9 ml supernatant per well). After 1 h at room temperature with occasional rocking, 3 ml of 1% agarose in MEM supplemented with 2% FCS was added to each well. The plates were incubated at 37 °C for 4 days before being fixed with 10% formalin in phosphate buffer (4 ml per well). After 1 h incubation at room temperature, the formalin and agarose were removed and the cell monolayers stained with 0·01% crystal violet

to visualize the plaques. Results are expressed as the number of p.f.u. of virus per foot-pad.

Results

There is a weak secondary CTL response in lymph nodes draining the site of HSV-1 infection

Naive C57BL/6 mice or animals that had been infected 6 weeks earlier were immunized with HSV-1 in the foot-pads and the lymph node-derived CTL response to the dominant gB determinant examined *ex vivo* from days 2 to 7 following infection using EL4 target cells pulsed with the gB peptide determinant (Fig. 1). The results show that some cytotoxicity activity can first be detected in the draining popliteal lymph nodes 3 days after infection and this activity peaks at day 5 in the primary response. In contrast, only weak cytotoxicity is evident in the lymph nodes throughout the total period from days 2 to 7 during the secondary response, with a low broad peak between days 3 and 4.

HSV-specific CTL can also be expanded *in vitro* without the addition of exogenous antigen (Nash *et al.*, 1980; Pfizenmaier *et al.*, 1977). To determine whether this expansion would reveal the presence of a more vigorous proliferative capacity for the recalled memory CTL, primary and secondary lymph node cells taken from mice challenged 2 to 7 days previously were placed in culture and the cytotoxicity was measured 3 days later on EL4 cells pulsed with gB peptide (Fig. 2). The results are expressed as lytic units per lymph node in an attempt to reveal any preferential expansion of CTL effectors in the secondary response. The *in vitro*-expanded cytotoxicity (Fig. 2) largely mirrors that seen directly *ex vivo* (Fig. 1), although the secondary response is a little more evident after the *in vitro* culture. Once again there is a relatively low peak of cytotoxicity between days 3 and 4 of the secondary response, while the somewhat larger primary response peaks between days 4 and 5 post-infection. Overall, the *in vitro* and *ex vivo* cytotoxicity data show surprisingly little evidence for any form of enhanced lymph node-derived secondary CTL response on rechallenge with HSV-1.

The gB-specific CTL activity mirrors the accumulation of CD8$^+$ T cell blasts expressing Vβ10$^+$ TCR

The weak cytotoxicity present in the secondary response could have simply reflected a lack of effector function rather than the absence of T cell accumulation or activation in the draining nodes. While unlikely, we excluded this possibility by taking advantage of the dominant TCR expression that forms a useful signature for presence of gB-specific CTL (Cose *et al.*, 1997). We have found a preferential accumulation of CD8$^+$ T

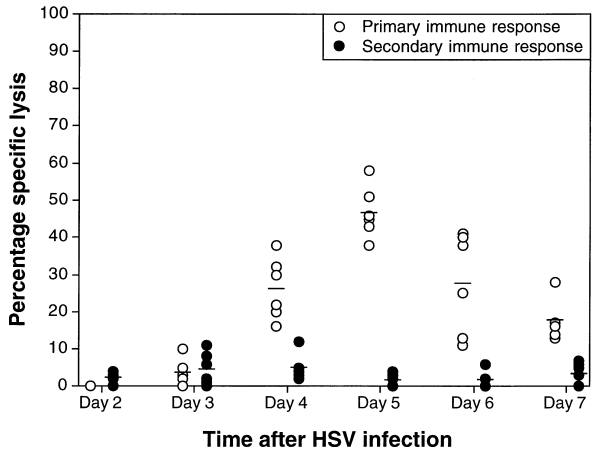

Fig. 1

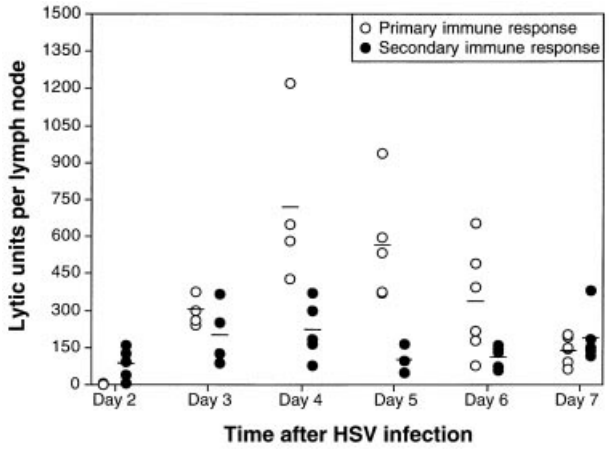

Fig. 2

Fig. 1. gB-Specific CTL activity in the draining lymph nodes in primary and secondary immune responses to HSV-1 infection. Mice were infected in the hind foot-pads with 4×10^5 p.f.u. HSV-1. Six weeks later these mice (●) and a group of naive mice (○) were infected with HSV-1 as before and after 2 to 7 days the cells from the popliteal lymph nodes were collected, viable cell counts were performed and the cells were assayed in a 4 h chromium release assay using ^{51}Cr-labelled EL4 cells in the presence or absence of 1 μg/ml gB peptide at varying effector-to-target ratios. Results are expressed as the percentage of specific lysis at an E:T ratio of 100:1. Spontaneous lysis was 13%. Each circle represents one lymph node preparation. The mean of each set of data points is indicated.

Fig. 2. Primary and secondary gB-specific CTL activity in draining lymph node cells following *in vitro* culture without exogenous antigen. Mice were infected in the hind foot-pads with 4×10^5 p.f.u. HSV-1. Six weeks later these mice (●) and a group of naive mice (○) were infected with HSV-1 as before and after 2 to 7 days the cells from the popliteal lymph nodes were collected, viable cell counts were performed and the cells were placed into culture for 3 days without exogenous antigen. One well from each culture was then serially diluted and assayed in a 4 h chromium release assay using ^{51}Cr-labelled EL4 cells in the presence or absence of 1 μg/ml gB peptide. Lytic units were determined for 20% specific lysis and the number of lytic units per lymph node was calculated. Each circle represents one lymph node preparation. The mean of each set of data points is indicated.

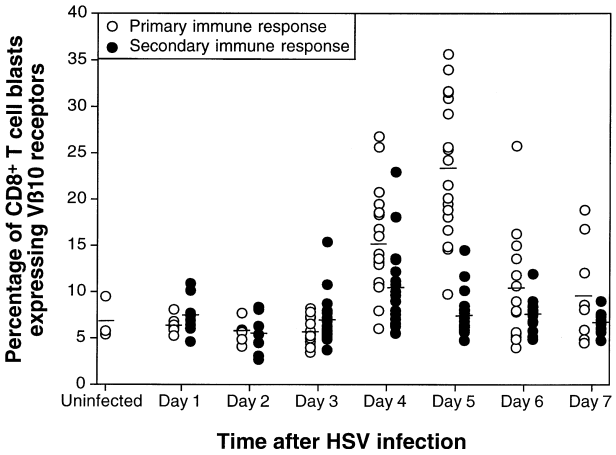

Fig. 3. Vβ10 expression by CD8$^+$ T cell blasts in the draining lymph nodes in primary and secondary immune responses to HSV-1 infection. Mice were infected in the hind foot-pads with 4×10^5 p.f.u. HSV-1. Six weeks later these mice (●) and a group of naive mice (○) were infected with HSV-1 as before and after 1 to 7 days the popliteal lymph nodes were removed and the cells were double-stained with Vβ10 and CD8 antibodies prior to analysis by flow cytometry. Cells were separated into small lymphocyte and large blast cell populations based on forward- and side-scatter. Results are expressed as the percentage of CD8$^+$ T cell blasts that express the Vβ10 receptor. Popliteal lymph node cells isolated from uninfected mice were used as a control. Each circle represents one lymph node preparation. The mean of each set of data points is indicated.

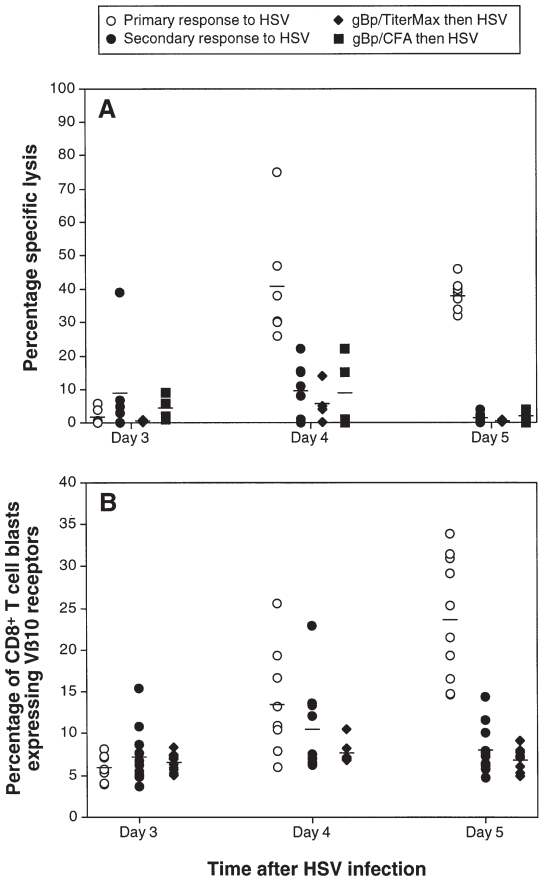

Fig. 4. Prior CTL priming results in the diminution of gB-specific CD8$^+$ T cells in the draining lymph nodes in the secondary response to HSV-1 infection. Mice were injected in the hind foot-pads with 4×10^5 p.f.u. HSV-1 (●) or with approximately 5 µg gB peptide (gBp) emulsified in either TiterMax (◆) or CFA (■). Six weeks later each group of mice and a group of naive mice (○) were infected in the hind foot-pads with 4×10^5 p.f.u. HSV-1 and after 3 to 5 days the cells from the popliteal lymph nodes were removed. Cells were assayed directly *ex vivo* for (A) gB-specific CTL activity on ^{51}Cr-labelled EL4 cells in the presence or absence of 1 µg/ml gB peptide, and (B) Vβ10 expression by CD8$^+$ T cell blasts. CTL assay results are expressed as the percentage of specific lysis at an E:T ratio of 100:1. Spontaneous lysis was less than 20%. Each circle represents one lymph node preparation. The mean of each set of data points is indicated.

cell blasts expressing a combination of dominant Vβ10 and junctional sequences in the popliteal lymph nodes draining HSV-1-infected foot-pads. The results in Fig. 3 track the preferential expression of Vβ10$^+$ TCR in CD8$^+$ blast cells isolated from the popliteal lymph nodes following HSV-1 infection for a large number of independent experiments. As found in our previous analysis, CD8$^+$ T cells expressing Vβ10$^+$ TCR peak at day 5 following primary infection. In this study only 23 ± 7% of these cells were found to be Vβ10$^+$ while in other experiments up to 45% of blast cells fall into this category (Cose *et al.*, 1997). Nonetheless, the results clearly show that the accumulation of Vβ10$^+$CD8$^+$ T cells in both the primary and secondary lymph nodes mirrors the cytotoxicity isolated from those sites. Again, while Vβ10-expressing TCR make up a significant proportion of the CD8$^+$ blasts at day 5 following primary infection, this proportion is much reduced in the secondary response where there is a clear, weaker peak at day 4. Consequently, it appears that prior exposure to HSV-1 results in a decrease in the accumulation of CD8$^+$ CTL specific for the dominant gB-determinant within the popliteal lymph nodes.

Prior CTL priming solely determines the reduced level of gB-specific CD8$^+$ T cells in the draining lymph nodes in the secondary response

Prior immunization with whole virus might result in preferential entry of CTL specific for determinants other than gB into the memory pool that are then expanded during the recall response. To eliminate this possibility we primed mice with the isolated gB peptide in adjuvant as well as with whole virus and then rechallenged these animals 6 weeks later with HSV-1 to recall the memory CTL. Examination of the cytotoxicity isolated directly *ex vivo* following virus challenge shows a diminution in the secondary gB-specific CTL response even with the priming focused totally on this determinant as a consequence of peptide immunization (Fig. 4A). This was true whether the peptide was injected emulsified in either TiterMax adjuvant or CFA, both of which provide effective peptide-specific CTL priming (Bennett *et al.*, 1997; Dyall *et al.*, 1995). The reduction in cytotoxicity is mirrored by the suppressed accumulation of CD8$^+$ T cell blasts preferentially expressing Vβ10$^+$ TCR within the lymph nodes (Fig. 4B). The peptide-

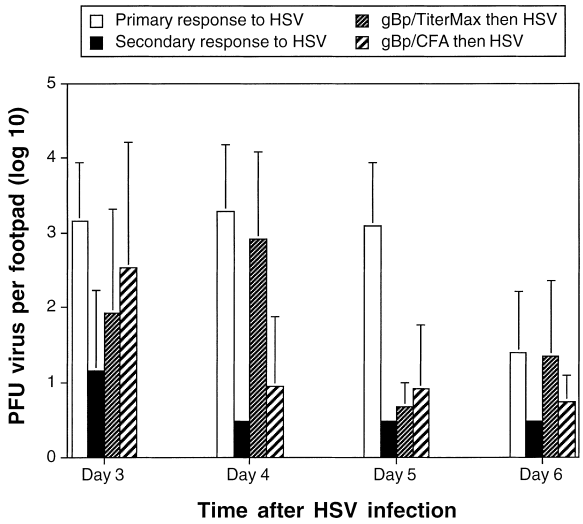

Fig. 5. Virus clearance at the site of infection in primary and secondary immune responses to HSV-1. Mice were injected in the hind foot-pads with 4×10^5 p.f.u. HSV-1 (■) or with approximately 5 μg gB peptide (gBp) emulsified in either TiterMax (▨) or CFA (▨). Six weeks later each group of mice and a group of naive mice (□) were infected in the hind foot-pads with 4×10^5 p.f.u. HSV-1 and after 3 to 6 days the hind feet were removed and homogenized. The resulting supernatant fluid was used in a p.f.u. assay on Vero cells to determine the virus titre. The results are expressed as the number of p.f.u. of virus per foot-pad. Each bar represents the mean and standard deviation of eight individual foot-pads.

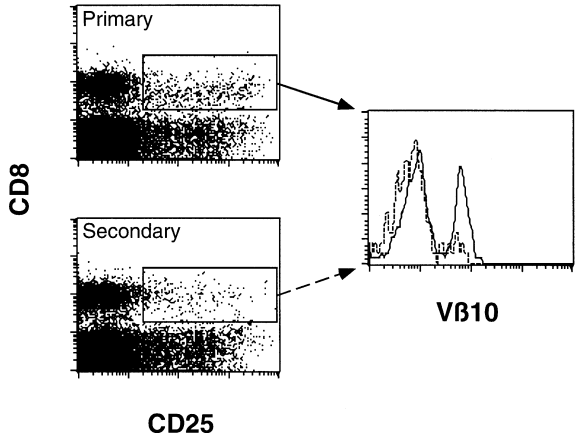

Fig. 6. Paucity of $CD8^+CD25^+$ cells expressing Vβ10 in the draining lymph node 5 days after re-infection with HSV-1. A C57BL/6 mouse was infected in the hind foot-pads with 4×10^5 p.f.u. HSV-1. Six weeks later this mouse and a naive C57BL/6 mouse were infected with HSV-1 as before and after 5 days the popliteal lymph nodes were removed and the cells were triple-stained with Vβ10, CD25 and CD8 antibodies prior to analysis by flow cytometry. Dead cells were excluded using propidium iodide staining. The dot plots represent CD8 versus CD25 staining for the popliteal lymph node cells from the primary and secondary immune mice. The histogram shows the Vβ10 receptor expression of the $CD8^+CD25^+$ cells from the primary (solid line) and secondary (dotted line) immune mice.

induced diminution of the secondary responses is at least as pronounced as that achieved by prior virus infection in both the parameters examined at the peak of the primary response (day 5). Despite the lack of gB-specific CTL in the draining lymph nodes, secondary CTL responses in mice primed with whole virus or with gB peptide in adjuvant were comparable at accelerating virus clearance. In all cases, negligible levels of HSV-1 were present in the foot-pads 3 to 4 days after secondary challenge in comparison with primary infection, where no virus clearance was observed until after day 5 post-infection (Fig. 5). Consequently, the reduction in the CTL response found in the secondary lymph nodes is solely dependent on prior gB-specific CTL activation and this priming enhances virus clearance on subsequent challenge.

A second wave of activated $V\beta10^+CD8^+$ T cells appears in the draining lymph nodes some time after virus clearance

The rapid virus clearance following secondary challenge in combination with the diminished CTL response in the popliteal lymph nodes suggested that the memory CTL might be directly activated within the infected tissues (i.e. the foot-pad). To date we have found it difficult to visualize $V\beta10^+$ CTL accumulation within this site. However, we reasoned that if such cells were present in the foot-pad, they would ultimately drain to the popliteal lymph nodes via the afferent lymphatics after the resolution of infection. In order to track the appearance of these specific CTL, we took advantage of IL-2 receptor α-chain (CD25) expression as a marker for recent activation, rather than cell size. The plot of CD25 versus CD8 expression is shown in Fig. 6 for primary and secondary popliteal lymph node cells 5 days after foot-pad immunization with HSV-1. This figure clearly highlights significant $V\beta10^+$ T cell accumulation at day 5 post-infection for the primary immune response and agrees with previous analyses using cell size as an activation marker (Fig. 3; Cose *et al.*, 1997). Moreover, Fig. 6 also shows a relative paucity of activated $CD8^+$ T cells in the secondary response which is combined with a diminished level of $V\beta10^+$ TCR expression within this subset. A more extensive time study presented in Fig. 7 reveals a discernible second wave of preferential accumulation of $V\beta10^+$ T cells at times past day 7 post-inoculation. This accumulation peaks at day 8 for the secondary response with $17 \pm 7\%$ of $CD8^+CD25^+$ lymph node cells from previously infected mice expressing Vβ10 compared to $12 \pm 5\%$ from animals undergoing a primary response. This preferential accumulation is specific for the popliteal lymph nodes since only $6 \pm 2\%$ of mesenteric lymph node $CD8^+CD25^+$ T cells from these animals expressed this V-region, which is close to the average found in unimmunized controls ($7 \pm 2\%$). This shows that the day 8 accumulation of $V\beta10^+$ T cells is localized to the draining popliteal lymph nodes and is not due to some general lymph node recirculation effect late in the immune response. Preferential accumulation of activated $V\beta10^+$ T cells is also found in the primary response at day 10 after immunization with $20 \pm 7\%$ of $CD8^+CD25^+$ lymph node cells from primary mice expressing Vβ10 compared to $9 \pm 6\%$ from secondary animals.

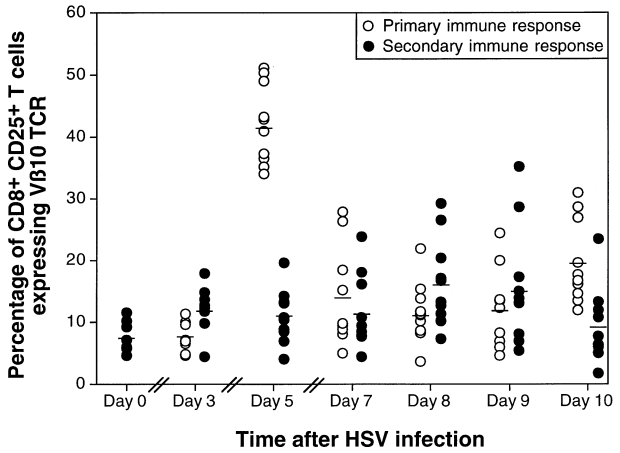

Fig. 7. Extended time-course of Vβ10 expression by $CD8^+CD25^+$ activated T cells in the draining lymph nodes in primary and secondary immune responses to HSV-1 infection. Mice were infected in the hind foot-pads with 4×10^5 p.f.u. HSV-1. Six weeks later these mice (●) and a group of naive mice (○) were infected with HSV-1 as before and after 3 to 10 days the popliteal and mesenteric lymph nodes were removed and the cells were triple-stained with Vβ10, CD25 and CD8 antibodies prior to analysis by flow cytometry. Dead cells were excluded using propidium iodide staining. Results are expressed as the percentage of $CD8^+CD25^+$ popliteal lymph node cells that express the Vβ10 receptor. Day 0 cells are $CD8^+CD25^+$ popliteal lymph node cells taken from primary infected mice that had been rested for 6 weeks. Each circle represents one popliteal lymph node preparation. The mean of each set of data points is indicated. Vβ10 receptor expression on $CD8^+CD25^+$ mesenteric lymph node cells was $6 \pm 2\%$ for the days examined.

Discussion

Priming of C57BL/6 mice with HSV-1 results in accelerated clearance on subsequent reinfection with this same virus. This is one of the hallmarks of adaptive immunity. Recent studies have clearly shown that increased precursor cell numbers expanded during the primary infection with either virus or bacteria directly contribute to the enhanced recall CTL response (Busch *et al.*, 1998; Flynn *et al.*, 1998; Murali-Krishna *et al.*, 1998). Consequently, it was initially surprising that HSV-1 reinfection of previously primed animals did not result in an increased cytolytic activity within the draining popliteal lymph nodes. This diminution was seen in both the level of cytotoxicity and the accumulation of $V\beta10^+$ TCR in the $CD8^+$ blast cell subset. While modern means of direct specificity detection, such as TCR staining using tetrameric MHC–peptide complexes (Altman *et al.*, 1996), provide comprehensive analyses of antigen-specific CTL (Doherty, 1998), biased Vβ expression, where present, represents a simple and reliable assessment of specific T cell responses as shown in other systems (Maryanski *et al.*, 1996; McHeyzer-Williams & Davis, 1995; Walker *et al.*, 1995). It provides a reliable means of tracking gB-specific CTL (Cose *et al.*, 1997) and argues that the decreased cytotoxicity evident in the secondary response was not simply a consequence of some unusual delay in expression of the lytic machinery but was rather a direct reflection of a lack of gB-specific $CD8^+$ T cell accumulation within the lymph nodes draining the site of HSV-1 infection.

Given the complexity of the HSV genome it was possible that the secondary response was simply directed to determinants other than gB. This was raised in a previous study which found a reduction in precursor CTL numbers during the peak of the recall response to this virus (Nugent *et al.*, 1994). However, analysis of multideterminant lymphocytic choriomeningitis virus (LCMV) and *Listeria* responses showed no significant changes in the hierarchy of immunodominance when comparing primary and secondary CTL populations (Busch *et al.*, 1998; Murali-Krishna *et al.*, 1998). Moreover, while differential specificity selection could occur in convalescent animals infected with HSV-1 this would seem unlikely for the gB peptide-immunized mice. Only gB-specific CTL precursors were primed in this situation and any diminution seen on subsequent infection would be a direct consequence of this initial priming.

Rather than affecting the bias in determinant immunodominance as a consequence of prior antigen exposure, we favour the notion that the initial priming changed the pattern of gB-specific CTL migration and it was this altered migration that directly, or indirectly, diminished the secondary lymph node response. Naive and memory T cells possess differential migratory properties, with memory lymphocytes in particular preferentially entering infected parenchymal tissue and then returning to the circulation via the afferent lymph (Mackay, 1991; Mackay *et al.*, 1990). This differential migration is combined with a relaxed requirement for co-receptor engagement (Croft *et al.*, 1994; Luqman & Bottomly, 1992; McKnight *et al.*, 1994) and, at least for memory CTL, a capability to kill targets in the absence of any further stimulation (Lalvani *et al.*, 1997; Zimmerman *et al.*, 1996). Consequently, on virus challenge memory T cells should be capable of directly dealing with tissue infection without prior activation within the lymphoid organs. Once the peripheral infection is resolved the specific CTL would be expected to either die *in situ* or return to the circulation via the draining lymph nodes. It is this latter post-infection and post-activation migration that most likely contributes to the transient increase in $V\beta10^+$ T cells found in the activated $CD8^+$ subset relatively late in both the primary and secondary responses to infection. These are assumed to be gB-specific CTL returning via afferent lymphatics.

Differential migration between naive and memory CTL alone, however, would not explain the diminution of lymph node-specific, gB-specific CTL accumulation during the secondary response. One possibility is that the memory CTL remain in the lymph node for a much shorter period prior to migration to the tissue. Alternatively, it is possible that rapid clearance of virus within the convalescent animals mediated by tissue activated memory CTL, $CD4^+$ T cells or antibody simply limits the available antigen and thus diminishes the resultant CTL activation seen in the draining lymph node.

While this seems feasible, it should be noted that more rapid antigen clearance did not appear to suppress other recall responses. Early control of virus and bacteria replication in influenza virus, LCMV and *Listeria monocytogenes* infections did not limit the massive CTL expansion evident in the recall response to these micro-organisms (Busch *et al*., 1998; Flynn *et al*., 1998; Murali-Krishna *et al*., 1998). In these studies, CTL numbers rose rapidly after reintroduction of the priming agent and persisted at high levels for some time after infection had been resolved. Unlike the LCMV and *Listeria* models of infection, HSV does not replicate within the site involved in the actual priming event. Consequently, the rapid and enhanced expansion of splenic CTL seen on LCMV and *Listeria* challenge could have been due to local proliferation of effector cells in order to deal with micro-organism replication rather than the initial activation of either the naive or memory CTL per se. For this model of HSV infection, priming occurs within the popliteal lymph nodes, while virus replication is confined to the essentially separate peripheral foot-pad compartment (Cook & Stevens, 1973; Cose *et al*., 1997).

The results presented here are consistent with the notion that the initial HSV-specific CTL priming occurs within the lymph nodes draining the site of infection (Carbone *et al*., 1998; Heath *et al*., 1998). Presentation would then be mediated by professional antigen presenting cells (APCs), which provide all the co-receptor ligands necessary for effective naive percursor cell priming. Memory CTL have a more relaxed co-receptor and APC requirement and therefore should no longer need the specialized cellular environment found within the draining lymph node. Consistent with peripheral activation for memory CTL, initial T cell priming within primary responses results in the rapid downregulation of CD62L, which promotes extravasation across high endothelial venules (Hamann *et al*., 1988; Jung *et al*., 1988). We found previously that the lymph node-derived Vβ10$^+$ blast cells bearing the gB-specific receptors were indeed CD62Llo at the peak of the primary response (Cose *et al*., 1997). While some antigen-specific memory CTL regain the CD62L marker (Busch *et al*., 1998; Tripp *et al*., 1995; Zimmerman *et al*., 1996), others maintain their CD62Llo phenotype for considerable periods (Doherty *et al*., 1996). The basis for the variable reacquisition of this receptor is unknown. Virus or antigen persistence, especially in peripheral or extra-lymphoid compartments, may favour the retention of a more activated or migratory CD62Llo phenotype. This would appear attractive given the persistent nature of HSV and the fact that one of the major functions of adjuvant emulsions is to form a long-lived antigen depot. If this were the case, then more transient means of antigen delivery might alter the pattern of memory T cell migration to include entry into the lymph nodes and permit subsequent reactivation within this site during the recall response.

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