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Murine epidermal antigen-presenting cells in primary and secondary T-cell proliferative responses to herpes simplex virus *in vitro*

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SUMMARY

The role of epidermal Langerhans' cells in infection with herpes simplex virus (HSV) was investigated using a culture system that supports antigen-specific primary and secondary T-cell proliferative responses. Epidermal cell suspensions were capable of restimulating the response of *in vivo* primed T cells to UV-inactivated HSV. This capability was also present in cell suspensions enriched for Langerhans' cells, but was abrogated by the depletion of I-A-bearing cells. The magnitude, kinetics and phenotype of the responding cells were similar to those elicited when HSV was presented to primed T cells by antigen-presenting cells from the spleen. In marked contrast, whereas splenic antigen-presenting cells induced strong antigen-specific proliferation of unprimed T cells (primarily of the helper phenotype), Langerhans' cells failed to invoke any detectable reaction of such cells.

INTRODUCTION

Herpes simplex virus (HSV) has long been a focus of attention for scientists since it not only causes an acute primary infection but also persists in the host as a latent infection.[1] Under certain circumstances the latent virus is reactivated and spreads back to the periphery, where it may again cause pathology, which is seen clinically as recurrent disease.[2] Hence there is a primary exposure of the host to viral antigens during the initial infection and repeated 'secondary' exposures whenever the latent virus is reactivated. To clarify the mechanisms involved it is therefore important to fully understand the immune responses which are stimulated during both primary and recurrent disease.

It is clear that upon secondary challenge, the immune response to the majority of antigens differs markedly from that which occurs after primary contact. This has long been known since secondary responses are usually more vigorous and occur with greater rapidity than primary reactions. However it is also evident that these changes are associated with phenotypic alteration of the lymphocytes which are available to react to the particular antigen. There is a shift in the immunoglobulin expressed on the membrane of the B cells,[3] and phenotypic changes also appear to occur on T cells.[4,5] Thus, in man, unprimed T cells express the high molecular weight (MW) form of the leucocyte common antigen CD45, whereas primed T cells bear the smaller 180,000 MW isoform.[4] Similar observations have been reported with rat T cells.[5] In addition to changing the expression of cell-surface markers, T-cell priming appears to be associated with a change in the requirement for antigen presentation. Thus it appears that while a wide variety of Ia-expressing cell types can reactivate immune T cells, only a few such cells appear to be able to prime effectively. For example, while resting B cells are able to restimulate immune T cells efficiently, they do not appear to be capable of initiating reactivity in naive animals.[6] In contrast, splenic dendritic cells are able to activate both immune and non-immune T cells efficiently.[7] Therefore, in studying immune mechanisms in primary herpetic infections it is not possible to extrapolate from data obtained using primed T-cell populations.

Since the epidermis is the major site of infection with HSV, it is important to elucidate the role of epidermal antigen-presenting cells (APC) and Langerhans' cells (LC) in the induction and restimulation of immune reactions to the virus. These cells can take up antigen *in vivo* and constitutively express products of the class II major histocompatibility complex (MHC).[11] Epidermal cell suspensions are capable of inducing the proliferation of primed T cells *in vitro* in response to a variety of specific antigens, including HSV.[7,12–15] This activity can be abrogated by the depletion of Ia-bearing cells[12,15] and is also supported by cell suspensions enriched for LC.[7,14] These observations indicate that the LC themselves provide the antigen-presenting capacity of epidermal cell suspensions. However, unless cultured for several days, LC appear to be very poor stimulators of unprimed T cells in mixed lymphocyte reactions (MLR) and antigen-specific plaque-forming cell assays.[7] These observations suggest that resting epidermal APC are not capable of activating unprimed T cells. It is therefore clear that in order to understand

Abbreviations: APC, antigen-presenting cell; BSA, bovine serum albumin; EC, epidermal cell; HSV, herpes simplex virus; LC, Langerhans' cell; MLR, mixed lymphocyte reaction; PBS, phosphate-buffered saline; PFU, plaque-forming unit; RC, rabbit complement.

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the events associated with infection with HSV the nature of the primary and secondary responses to the virus needs to be established. In particular, it is necessary to assess directly whether cells from the epidermis are capable of stimulating protective responses.

MATERIALS AND METHODS

Animals

The animals used were 8–10-week-old female CBA/Ca mice, bred in the departmental animal facility from stock originally purchased from the National Institute of Medical Research (Mill Hill, London, U.K.). All animals were age matched within each experiment.

Virus

A strain of HSV-1, strain SCI6, originally isolated from a labial lesion at the PHLS, Bristol, U.K.[15] was used throughout this investigation. In order to avoid cross-reactivity, virus stocks for immunization of animals were prepared in Hep-2 cells, whereas stocks for use *in vitro* were grown in Vero cells. Titres of HSV were assessed by plaque assay. Virus was inactivated for use *in vitro* by exposure to a lethal dose of ultraviolet light. The efficacy of the inactivation procedure was routinely tested by plaque assay.

Immunization

Mice were immunized subcutaneously in the flank with 1×10^5 plaque-forming units (PFU) of live virus 10 days prior to initiation of lymphocyte cultures.

Cell preparation

Spleens were excised aseptically and minced through stainless steel mesh into phosphate-buffered saline (PBS). Debris was left to settle for 5 min and the cell suspension was decanted and washed three times with PBS by centrifugation at 300 *g*. T cells were purified by passage of spleen cell suspensions through rabbit anti-mouse immunoglobulin (Ig)-mouse Ig-coated glass-bead affinity columns.[16] The cells collected were greater than 95% T cells, as defined by immunofluorescent staining with anti-Thy-1 antiserum assessed by flow cytometry using an EPICS (Coulter, Luton, Beds, U.K.) (data not shown).

APC (always from non-immune syngeneic mice) were unselected spleen cells, unselected epidermal cells (EC) or LC purified on a density gradient. These cells were usually used as viable cells but in some cases they were treated with 1500 rads from a cesium source (Gravatom Industries Ltd, Gosport, Hampshire, U.K.). Irradiation did not appear to affect the responses (data not shown). EC were dissociated from the pinnae of mice using a method adapted from that of Stingl *et al.*[17] In brief, split pinnae were incubated in 4 mM ethylenediamine tetra-acetic acid (tetrasodium salt; Sigma Chemical Company, Poole, Dorset, U.K.) in PBS for 2 h at 37° in a humidified environment of 5% CO_2 and 95% air. The epidermis was removed from the dermis and the epidermal sheets were floated on 1% trypsin (Difco, Detroit, MI) in PBS for 75 min at 37° in the incubator. The resulting cell suspension was washed at least three times by centrifugation in PBS before culture. LC were enriched from EC at the medium/14·5 w/v interface of a metrizamide (Nycomed, Nyegaard & Co. AS, Oslo, Norway) density gradient.[18] The cells were layered on to the metrizamide at $4-8 \times 10^6$/ml and spun at 600 *g* for 10 min. When assessed immediately after collection, 32·5% of the cells found at the interface of the medium and metrizamide could be stained with anti-I-A using a PAP technique but 85·3% were positive following 48 hr in culture. In contrast, no I-A-bearing cells were found in the pellet.

The viability of all the cell suspensions was assessed by their ability to exclude the dye trypan blue. Cell preparations with viability lower than 90% were discarded.

Cultures

The culture medium was the α-modification of Eagles' minimal essential medium (Flow Laboratories, Irvine, Ayrshire, U.K.; Gibco Europe Ltd., Paisley, Renfrewshire, U.K.) supplemented with 4 mM L-glutamine (Gibco), 5×10^{-5} M 2-mercaptoethanol (Sigma), 100 U/ml benzyl penicillin (Glaxo Ltd, Greenford, Middlesex, U.K.), 20 mM HEPES (Sigma), and 0·5% fresh autologous normal mouse serum. Therefore no heterologous serum additives were present in the cultures.

Primary cultures contained $1{\cdot}25 \times 10^6$ non-immune T cells/ml in either 2-ml volumes in 24-well plates (Nunc A/S, Roskilde, Denmark), or in 8–10-ml volumes in 50-ml flasks (Falcon, Oxford, Oxon, U.K.). Secondary cultures of *in vitro* primed T cells were carried out in 0·2-ml volumes in 96-well microtitre plates (Sterilin Ltd, Hounslow, Middlesex, U.K.) using a concentration found to be optimum for responses ($0{\cdot}3 \times 10^6$ cells/ml). Secondary cultures of *in vivo* primed T cells were carried out similarly except that the optimum T-cell concentration was $1{\cdot}25 \times 10^6$/ml. Splenic APC were, unless stated, used at concentrations between 0·8 and $1{\cdot}0 \times 10^6$ cells/ml. EC were usually used at the concentrations found to be optimal ($2{\cdot}5 \times 10^5$ cells/ml with primed T cells, and at 4×10^5 cells/ml with T cells from non-immune mice).[19]

Cultures were incubated at 37° in a humidified atmosphere of 5% CO_2 and 95% air. After an appropriate period in culture, three replicate 0·2-ml microtitre cultures or 0·1-ml microtitre samples from 2- or 8-ml cultures were pulsed with 1 μCi per well of [^{3}H]thymidine (73–85 Ci/mM; NEN Research Products, Boston, MA) in 20 μl of medium. Following 6 h of further culture the samples were harvested onto glass fibre filter mats (Whatman Labsales Ltd, Maidstone, Kent, U.K.), and the [^{3}H]thymidine incorporated into newly synthesized DNA was measured by conventional liquid scintillation procedures in a LKB rackbeta (LKB-Wallac, Turku, Finland). Results are expressed as the mean counts per minute (c.p.m.) with the standard error of the mean of the replicate cultures. Statistical significance was evaluated by Student's *t*-test.

Cell depletion with antiserum and complement

Negative selection was carried out as a one-step procedure. Cells were incubated for 1 h at 37° with 0·5 ml of either medium, rabbit complement (RC; 1:10 dilution in medium, Low Tox-M; Cedarlane, Hornby, Ontario, Canada), or RC with antiserum. The specific monoclonal antisera used were anti-I-A^k (1:1000; Cedarlane), anti-Thy-1.2 (1:10,000; NEN), anti-Lyt-1.1 (1:10,000; NEN), anti-Lyt-2.1 (1:5000; NEN) and anti-L3T4 (1:1000; Seralab Ltd, Crawley Down, Sussex, U.K.). Treated cells were washed three times in PBS, resuspended in medium and counted. The cells treated with RC alone were counted and the volume was adjusted to produce the desired cell concentration. The remaining samples were made up to the same volume as the RC control so that the numbers of antibody-treated cells

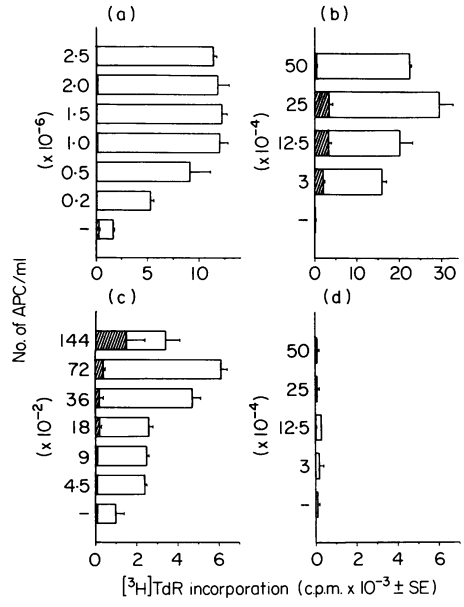

Figure 1. The peak proliferative response of immune T cells to HSV in the presence and absence of varying concentrations of different APC populations: (a) spleen cells, (b) epidermal cells, (c) epidermal suspensions enriched for LC, (d) suspensions of epidermal cells depleted of LC. $1{\cdot}25 \times 10^6$ T cells/ml were cultured with the indicated number/ml of added APC and either $1{\cdot}2 \times 10^7$ PFU/ml HSV (□) or no antigen (▨). Reactivity was maximal on Day 4 of culture in (a) and on Day 5 of culture in (b), (c) and (d).

added to the cultures reflected the loss of cells specifically sensitive to the treatments.

RESULTS

Presentation of HSV to primed T cells by splenic and epidermal cells

The addition of cells from either the spleen or the epidermis of naive animals to cultures of primed T cells resulted in a strong proliferative response, provided the immunizing antigen, HSV, was also present (Fig. 1a, b). The maximal response was obtained using $1{\cdot}0 \times 10^6$ spleen cells per ml (an APC to T-cell ratio of 1:1·25), whereas a concentration of $2{\cdot}5 \times 10^5$/ml (an APC to T-cell ratio of 1:5) elicited the highest response when epidermal cells were used. Figure 1 c shows that cell suspensions enriched for LC by density centrifugation were also capable of promoting a response to HSV. A significant response was attained when as few as $4{\cdot}5 \times 10^2$ LC were added per ml, although the reaction was optimal in the presence of $7{\cdot}2 \times 10^3$ LC/ml (an APC to T-cell ratio of 1:2780). In contrast, the EC depleted of LC, from the pellet following density centrifugation, were very poor stimulators of primed T-cell division (Fig. 1d).

The effect of depleting I-A-bearing cells from EC suspensions was assessed to test whether the secondary responses supported by EC were the result of class II MHC-restricted presentation (Fig. 2). Whereas untreated EC or those treated with complement alone supported similar strong cell division, EC depleted of I-A-bearing LC did not stimulate proliferation above background.

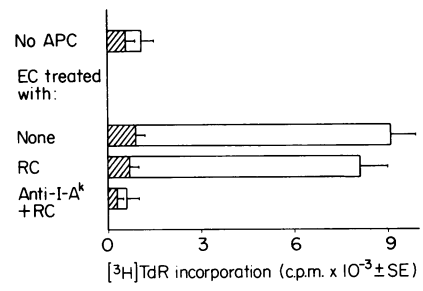

Figure 2. Peak proliferation (Day 5) in cultures of immune T cells (at $1{\cdot}25 \times 10^6$ cells/ml) with either $1{\cdot}2 \times 10^7$ PFU/ml HSV (□) or no antigen (▨). Cultures were performed in the absence of APC, and in the presence of epidermal cells ($2{\cdot}5 \times 10^5$ cells/ml or less) treated as indicated.

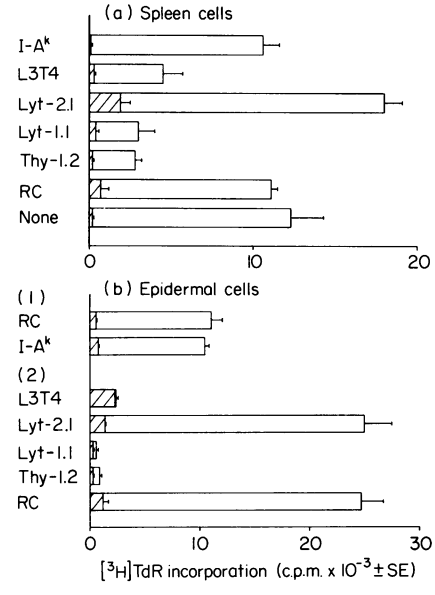

Figure 3. The phenotype of the HSV-responsive T cells stimulated in culture with HSV and splenic (a) or epidermal (b) APC. T cells from immune mice were treated with the indicated reagents and then cultured at $1{\cdot}25 \times 10^6$ cells/ml or less with either $0{\cdot}8 \times 10^6$ anti-Thy-1.2 plus RC-treated spleen cells/ml or $2{\cdot}5 \times 10^5$ epidermal cells/ml. Cultures were performed in the presence (□) or absence (▨) of $1{\cdot}2 \times 10^7$ PFU HSV/ml. The results of two separate experiments (as indicated) are shown in (b).

Phenotype of the HSV-reactive responder cells stimulated by splenic and epidermal APC

The previous data indicate that, like spleen cells, LC can support the proliferation of primed T cells in response to HSV *in vitro*. However, it is conceivable that the LC in the cultures are simply helping residual APC in the T-cell fraction to present antigen in a non-specific manner. Further, it is feasible that the cells responding in the cultures with EC are not relevant to the induction of an immune response *in vivo*. In order to test these possibilities the responder cells were depleted of specific cell populations prior to the initiation of the assay cultures (Fig. 3). The secondary response of T cells in the presence of splenic APC was greatly reduced following the removal of cells bearing Thy-1, Lyt-1 or L3T4. In contrast complement-dependent negative

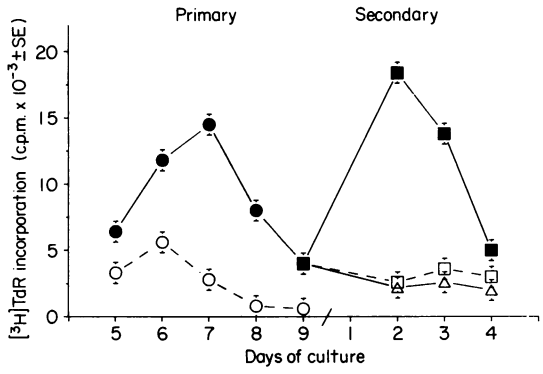

Figure 4. Primary and secondary proliferative responses of T cells to HSV utilizing splenic APC. Primary cultures contained $1{\cdot}25 \times 10^6$ T plus $0{\cdot}8 \times 10^6$ spleen cells/ml from non-immune mice without (○) or with (●) $1{\cdot}2 \times 10^7$ PFU/ml HSV. After 9 days in culture, cells were recovered and then restimulated at a concentration of $0{\cdot}3 \times 10^6$ cells/ml with fresh splenic APC ($0{\cdot}8 \times 10^6$ cells/ml). To restimulated cultures was added: no antigen (□), $1{\cdot}2 \times 10^7$ PFU/ml HSV (■), or mock HSV at a concentration equivalent to $1{\cdot}2 \times 10^7$ PFU/ml (△).

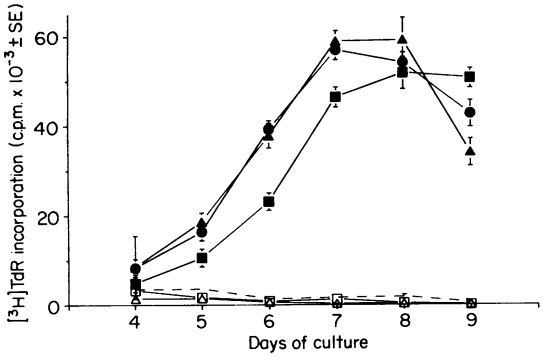

Figure 5. Primary proliferative response of T cells (at $1{\cdot}25 \times 10^6$ cells/ml or less) to HSV ($1{\cdot}2 \times 10^7$ PFU/ml) in the presence of $0{\cdot}8 \times 10^6$ anti-Thy-1·2 plus RC-treated spleen cells/ml. T cells were: untreated (▲), treated with RC alone (●), or treated with RC in conjunction with; anti-Thy-1·2 (□), anti-L3T4 (△) or anti-Lyt-2 (■) prior to initiation of the cultures. The highest response of any group in the absence of antigen is also shown (- -).

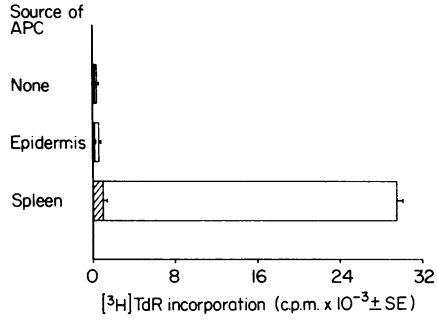

Figure 6. The peak proliferative response (Day 7) of non-immune T cells to HSV in the absence of APC or in the presence of APC from either the epidermis or spleen. T cells were cultured at $1{\cdot}25 \times 10^6$ cells/ml, spleen cells were at $0{\cdot}8 \times 10^6$ cells/ml, and epidermal cells were at $4{\cdot}5 \times 10^5$ cells/ml in the presence (□) or absence (▨) of $1{\cdot}2 \times 10^7$ PFU/ml HSV.

selection with anti-I-A^k had no effect, and depletion with anti-Lyt-2 significantly enhanced thymidine uptake. Similar analysis in the presence of EC showed that the reaction of primed cells was totally abrogated after removal of Thy-1, Lyt-1 and L3T4-expressing cells, whereas removal of Lyt-2 and, in a separate experiment, removal of I-A^k-bearing cells had no effect. Therefore the T cells proliferating in response to HSV both in the presence of splenic and epidermal APC were largely Thy-1^+, Lyt-1^+, L3T4^+, Lyt-2^-, I-A^-. Additionally the data show that the ability of EC to present HSV to primed T cells is independent of any residual I-A-bearing cells in the T-cell fraction.

Stimulation of HSV-specific primary proliferative responses by splenic APC

The addition of HSV to cultures of non-immune T cells and syngeneic unselected spleen cells induced a strong proliferative reaction which did not occur in cultures without antigen (Fig. 4). This primary response was apparent after 5 days of culture but did not become maximal until Day 7. To test whether the cell division observed in these primary cultures reflected the priming of HSV-specific T cells, cells were recovered on Day 9 and restimulated with fresh APC and antigens. The presence of a mock virus preparation did not elicit significant cell division above that which occurred in the absence of any antigen (Fig. 4). However, in cultures containing HSV a strong proliferative response occurred, which was maximal 2 days after restimulation. It is noteworthy that despite having approximately 1/4 the number of T cells as in the primary culture, the secondary reaction was of a similar magnitude to the primary, and reached its peak 5 days earlier.

Phenotype of the non-immune T-cell proliferating in response to HSV

The inability of the host to prevent the occurrence of recrudescent infections with HSV may in some cases be due to the stimulation of an inappropriate cellular response. The induction of such a response may be a property of the virus. To test this possibility the phenotypes of the cells reacting in primary cultures was investigated by the removal of specific populations prior to culture with splenic APC (Fig. 5). The response of naive T cells to HSV was totally abrogated by the complement-mediated depletion of cells bearing either Thy-1 or L3T4 antigens. In contrast, a strong response remained evident following removal of Lyt-2-bearing cells. However, despite reaching a similar peak level, the response in the anti-Lyt-2-treated cell cultures was initially lower than that in the control untreated and complement-alone treated cultures. Interestingly peak proliferation in cultures depleted of Lyt-2^+ cells was maintained as proliferation in the control cultures subsided.

Comparison of the capacities of splenic and epidermal APC to present HSV to unprimed T cells *in vitro*

Since primary herpes infections involve replication in peripheral tissues such as the epidermis it was important to determine whether the epidermis as a tissue possessed the ability to stimulate a response by unprimed T cells. Figure 6 shows the results of an experiment to determine whether EC can substitute for spleen cells as APC in cultures with unprimed T cells. In the

absence of added APC from either source the T cells did not respond significantly to HSV. As previously demonstrated the addition of unselected spleen cells elicited a strong reaction which did not occur if virus was not present. However, in cultures containing EC as the source of APC, T-cell division in the presence of HSV was not significantly higher than that which occurred in its absence.

DISCUSSION

Primary contact with an immunogen causes changes in the antigen-specific T-cell population which results in greatly enhanced reactivity upon secondary exposure to antigen. It is now becoming clear that these changes are associated with a shift in the phenotype of the T cells.[4,5] In addition it appears that the requirements of primed and unprimed T cells for antigen presentation may differ.[6,7] Therefore, in studying the immune response to an immunogen it is important to consider the reactions of both immune and non-immune T cells. Such considerations are particularly relevant for agents such as HSV, which avoids complete clearance from the host, and where responses are unable to prevent the occurrence of recrudescent infection. Therefore we have investigated the primary and secondary immune response to HSV *in vitro*, concentrating particularly on whether cells from the major site of infection, the epidermis, are able to present HSV to primed and unprimed T cells.

In secondary *in vitro* cultures, T cells isolated from the spleens of immune mice proliferated provided HSV and a source of APC were also present. Cells from both the spleen and the epidermis acted as accessory cells supporting responses of comparable magnitude, although differences were noted in the optimal APC:T-cell ratios required. Many cell types can present antigenic determinants in the context of class I MHC products; however, presentation associated with class II antigens is restricted to a very few.[20-22] Stimulation of T-helper cells only follows recognition of antigen in the cleft of class II molecules,[23] and hence it is such presentation that is important in the induction phase of the immune response. The following considerations indicate the relevance of the observed proliferation to the induction of *in vivo* immune responses: (i) evidence that the reaction was dependent on Ia-bearing cells, and was probably therefore class II-restricted, came from two studies. In the first it was shown that a population of EC which contained much increased numbers of I-A-expressing LC (from the interface of a density gradient) stimulated T-cell division when as few as 450 were added to $1{\cdot}25 \times 10^6$ T cells. In contrast, cells which passed through the gradient did not stimulate significant proliferation to HSV. Secondly, and more directly, cytotoxic depletion of I-A-bearing cells from EC suspensions totally blocked their ability to act as APC. Since LC are the only cells in the epidermis to constitutively express I-A antigens[11] this provides evidence that LC are the APC in this system. (ii) Pretreatment of T cells with anti-I-A antibody and complement to remove any remaining APC in the T-cell fraction did not significantly alter the proliferative response to HSV stimulated by EC. Therefore residual APC in the T-cell fraction were not simply being helped to present the virus in a non-specific manner, for example by the production of interleukin-1 (IL-1). (iii) Confirmation that the proliferation in the cultures was primarily of T-helper cells came from depletion studies. Thus the phenotype of the proliferating T cell stimulated by both spleen cells and EC was primarily Thy-1$^+$, Lyt-1$^+$, L3T4$^+$, Lyt-2$^-$; IA$^-$; the phenotype of the classical T-helper cell. It is noteworthy that cell division in cultures depleted of Lyt-2-bearing cells, in the presence of APC from either the spleen or the epidermis, was higher than in the control. This suggests that some stimulation of conventional Lyt-2$^+$ regulatory T cells may occur in the cultures.

The responses to HSV stimulated by splenic or epidermal APC were, however, only similar when the T-cell population was derived from primed animals. There were marked differences in the abilities of APC from the two tissues to induce the HSV-specific proliferation of non-immune T cells. In the presence of spleen cells, T cells isolated from non-immune mice mounted a strong proliferative response to HSV. Although detectable on Day 5 of culture this reaction did not become maximal until Day 7; some three days after the peak response of primed T cells. Following a decline in cell division on Days 8 and 9, cells from the cultures were restimulated with a fresh source of APC and either HSV, a mock viral preparation, an unrelated antigen (bovine serum albumin; BSA), or no antigen. The lack of response to either the mock antigen or to BSA (despite the fact that BSA is itself capable of stimulating a primary response in such cultures which is found to be BSA-specific upon restimulation),[19] together with the anamnestic characteristics of the reaction to HSV, provide strong evidence that virus-specific T-cell priming had occurred in the initial cultures. The primary proliferative response was shown to be relevant to the induction of the immune response *in vivo* since the reaction was dependent on the presence of a Thy-1$^+$, L3T4$^+$, Lyt-2$^-$ bearing cell type, and therefore probably results from division of T-helper cells. Interestingly, the response in the absence of Lyt-2-bearing cells was lower than that of the controls, yet no significant reaction occurred following removal of L3T4$^+$ cells. Thus it appears that some Lyt-2$^+$ cells proliferate in the cultures, but their proliferation requires the presence of L3T4$^+$ cells. The maintenance of peak proliferation in cultures depleted of Lyt-2$^+$ cells beyond that of the controls may be evidence that, as was observed in cultures of primed T cells, Lyt-2$^+$ cells have a regulatory role in the reaction.

Therefore it has been shown that the spleen harbours APC which are capable of presenting HSV to non-immune virus-specific T-helper cells *in vitro*. In marked contrast, when EC were substituted for spleen cells in these cultures no significant reaction to the virus was observed, despite the use of a wide range of EC concentrations. This suggests that the epidermis does not have the ability to prime HSV-specific T cells. It could be argued that this observation is an artifact produced by the vigorous isolation procedures required to attain cell suspensions from the epidermis. However, it is noteworthy that cells isolated by identical procedures from the spleen, Peyer's patches and lamina propria of the small intestine are all able to prime T cells (N. A. Williams, unpublished observations). If cultured for 72 h in the presence of heterologous serum and keratinocytes or granulocyte–macrophage colony-stimulating factor (GM-CSF), LC can subsequently stimulate primary allogeneic MLR and T-dependent antibody responses.[7] In contrast, LC isolated from EC suspensions after only 12 hr of preculture cannot then prime in either assay.[7] It has been suggested that maturation of LC in the EC cultures is associated with their acquiring the ability to bind T cells independently of antigen.[7] In our cultures

(which more closely mimick the situation in the epidermis since they contain keratinocytes throughout and are devoid of heterologous proteins and foetal growth factors) no such maturation is evident. It may be argued that the presence of EC other than LC in our primary cultures could inhibit the capacity of the LC to prime. Such an effect is unlikely to be mediated through inhibition of the LC since, as discussed, preculture of LC as EC suspensions appears to be a prerequisite to attaining the capacity to prime in other systems.[7] The possibility that cells in the EC fraction (perhaps Thy-1-positive dendritic cells) inhibit T-cell activation in our system nevertheless remains. We therefore conclude that while LC may acquire the capacity to prime T cells, either the necessary activation does not occur in cultures of EC and T cells in the absence of heterologous foetal serum, or T-cell activation is inhibited by other EC. It therefore seems unlikely that local activation of T-cell responses to HSV occurs in the epidermis during initial encounter with the virus. Primary contact with antigen *in vivo* causes migration of LC to regional lymph nodes. It is possible that the different environment encountered by LC outside the epidermis could allow them to become able to prime.

The inability of the epidermis to initiate quickly a specific T-cell response to HSV *in situ* may allow the virus longer to establish infection in the skin and thereby spread to the sensory nervous system. Indeed, in zosteriform lesions in mice cellular infiltration of the infected epidermis only occurs once virus spreads as far as the dermis.[24] However, such a delay may be necessary to avoid the initiation of an entirely local cellular response to the exclusion of a more multi-faceted reaction which would follow priming more centrally. Nevertheless, it is evident, from these and other studies,[7,12–15] that upon secondary challenge, for example as reactivated virus enters peripheral tissues, LC can very efficiently present antigen to primed T cells and thereby control the development of recurrent infection. However, the fact that recrudescent disease does sometimes occur suggests that the barrier provided by the LC activity must, in some cases, be compromised by some additional factors. In this respect we have reported elsewhere that the ability of drugs to cause recrudescence of experimental infection in mice closely correlates with their effectiveness at reducing LC function *in vitro*.[25]

ACKNOWLEDGMENTS

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