Cell-mediated Immunity in Herpes Simplex Virus-infected Mice: Suppression of Delayed Hypersensitivity by an Antigen-specific B lymphocyte

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SUMMARY

Lymph node cells, regional to the site of infection with herpes simplex virus type I (HSV-I), when taken 1 to 9 months p.i. and transferred to HSV-I immune recipients, suppressed the delayed type hypersensitivity (DTH) reaction to the virus. The suppressive activity was specific for HSV-I and was transferred by a thy-1.2-negative, Ig-positive, lymphocyte population. Anti-HSV-I serum did not suppress the HSV-I-induced DTH response. Contralateral lymph nodes contained little or no suppressor cell activity but in infected, adult thymectomized mice, these lymph node cells were as effective as the draining node in transferring suppression. The significance of these observations for the pathogenesis of herpes infections is discussed.

INTRODUCTION

The realization that cells of the immune system could impose a state of unresponsiveness or control on immune responsiveness has captured the attention of cellular immunologists in recent years (Gershon, 1975). Most areas of immunity, whether antibody or cell-mediated can involve a suppressor cell which regulates or competes with a corresponding effector cell (Cantor et al., 1976; Taylor & Basten, 1976). The major cell type that mediates the suppressor function in a specific or non-specific manner is a T lymphocyte. In the mouse, this cell is characterized as Lyt 2,3-positive (Cantor et al., 1976) and has antigens coded by the I-J subregion of the H-2 complex (Murphy et al., 1976).

The control mechanisms in delayed type hypersensitivity (DTH) reactions have been described for contact-sensitizing agents such as picrylsulphonic acid (Zembala & Asherson, 1973), azobenzenarsenate-coated lymphoid cells (Bach et al., 1978) and antigens such as horse gamma-globulin (Miller et al., 1979). In all cases the suppressor cell is thymus-derived. However, B suppressor cells have also been described against some forms of DTH reactions in guinea-pigs and mice (Turk et al., 1976).

In the preceding paper we have described the induction and characterization of DTH to HSV-I in mice. It was suggested from this work that cells obtained from the draining lymph node (DLN) at 'late' times (28 days p.i.) may contain some suppressors of cutaneous hypersensitivity. In this paper we describe a suppressive effect on DTH to HSV-I which appears to be mediated by B lymphocytes.
Methods

Mice, virus strains, inoculation of virus, lymph node cell preparations, anti-thy-1.2 serum treatment and the separation of Ig-positive and Ig-negative cell populations is as described in the preceding paper (Nash et al. 1980).

Lymphoid cell transfers and measurement of ear swelling. The basic procedure was as described in the preceding paper, except that all recipients of lymphoid cells were Balb/c mice sensitized with HSV-1 or vaccinia, 1 to 2 months previously. Ear thickness was measured as described in the preceding paper.

Adult thymectomy. This was carried out at 6 to 7 weeks old by standard methods using hypnorm–valium anaesthesia. The completeness of the operation was checked macroscopically at necropsy.

Results

Detection of suppressors of DTH in draining lymph nodes

Draining lymph node (DLN) cells, obtained between 16 days and 3 months p.i. were transferred to recipient mice, simultaneously injected with 10^4 p.f.u. of HSV-1, and the effect on the DTH response measured after 24 h and on successive days. Of 17 experiments performed 13 were positive in demonstrating a reduction in DTH ranging from 30 to 60%. An example of a suppression of the DTH response is shown in Fig. 1. In this and subsequent experiments non-immune lymph node cells failed to suppress DTH.

As seen in Table 1 the suppression of DTH was a feature of the draining lymph node cells; the contralateral lymph node (CLN) cells were largely ineffective in the suppressor activity.

Specificity of the suppression of DTH

The specificity of the suppressor cells was investigated using vaccinia-infected recipients. In the experiment shown in Table 2 DLN cells from HSV-1 immune mice were transferred...
Table I. Comparison of contralateral LN cells and draining LN cells on the suppression of DTH responses

<table>
<thead>
<tr>
<th>Expt.</th>
<th>Previously infected (months)</th>
<th>CLN*</th>
<th>DLN*</th>
<th>Control (no cells)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3</td>
<td>23±2</td>
<td>14±6</td>
<td>22±5</td>
</tr>
<tr>
<td>2</td>
<td>4</td>
<td>19±4</td>
<td>12±6</td>
<td>21±5</td>
</tr>
<tr>
<td>3§</td>
<td>1</td>
<td>36±4</td>
<td>19±5</td>
<td>37±2</td>
</tr>
<tr>
<td>4</td>
<td>2</td>
<td>14±5</td>
<td>10±0</td>
<td>18±6</td>
</tr>
</tbody>
</table>

* 1.6×10^7 to 2×10^7 CLN or DLN cells transferred/mouse.
† Mean±S.D. of three to four mice/group.
‡ Numbers in bold type indicate significant differences when compared to control values (P between 0.025 and 0.001).
§ 5×10^4 p.f.u. HSV-1 inoculated.

Table 2. Specificity of the suppressor cells for HSV-1

<table>
<thead>
<tr>
<th>Previously infected with</th>
<th>Challenge virus</th>
<th>Experimental group* (cells transferred)</th>
<th>Increased ear thickness† (mm×10^-3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HSV-1 (10^5 p.f.u.)</td>
<td>HSV-1</td>
<td>Control (no cells)</td>
<td>28±3.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>HSV-1-immune cells</td>
<td>17±6†</td>
</tr>
<tr>
<td>Vaccinia (10^4 p.f.u.)</td>
<td>Vaccinia</td>
<td>Control (no cells)</td>
<td>20±2.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>HSV-1-immune cells</td>
<td>18±6</td>
</tr>
</tbody>
</table>

* Both HSV-1 and vaccinia immune mice received 2×10^7 DLN cells from mice infected 2 months previously.
† Mean±S.D. of five to six mice/group.
‡ Numbers in bold type indicate significant differences between control and cell transfer (P > 0.001).

into groups of recipients previously infected with either HSV-1 or vaccinia and the effect on the DTH response to the respective viruses measured. Clearly the HSV-1 immune cells did not suppress the DTH response to vaccinia but produced up to 40% suppression of the DTH response to HSV-1.

Characteristics of the cell transferring suppression

Investigations were carried out to identify which population of cells was transferring the suppression. In the first series of experiments DLN cells from mice inoculated with virus, 1 to 2 months previously, were treated with anti-thy-1 and complement and transferred to immune recipients. In Table 3 it is evident that anti-thy-1 treatment did not abolish the suppressor effect. It was particularly interesting to observe that under conditions in which untreated DLN cells failed to transfer suppression, treatment with the anti-thy-1 serum and complement did produce a population which had suppressive activity (Table 3a, Expt. 2). These experiments suggest that the suppressor cell may have B cell properties.

To investigate this further, DLN cells were separated into Ig-positive and Ig-negative fractions using SpA-anti-mouse poly(Ig)-coated dishes. The Ig-positive populations were readily recovered from the dish surface, and transferred into immune recipients. Table 3(b) shows two experiments in both of which the Ig-positive fraction transferred suppression. Expt. 2, Table 3(b) was similar to Expt. 2, Table 3(a), in that the unseparated cells and Ig-negative fraction failed to transfer suppression but the Ig-positive fraction was effective.

These experiments clearly define a population of B cells present in the DLN of long-term
immune mice that exert a suppressive activity on DTH response to HSV-1. It is also apparent that in two experiments where the untreated DLN cells failed to transfer a suppressive effect, removal of T cells allowed the suppressor B cell population to function.

One possible way in which the B cell suppression could be working is by production of antibody to HSV-1. However, in experiments where Balb/c anti-HSV-1 serum was transferred to HSV-1 immune mice no reduction in the DTH response to HSV-1 was observed (Table 4). It therefore appears unlikely that antibody is directly involved in the suppressive effect and other mechanisms need to be explored.

### Effect of adult thymectomy on the DTH suppressor system

HSV-1 immune mice were thymectomized at 7 weeks old (3 weeks p.i. with $10^6$ p.f.u. HSV-1) and the LN cells removed 2 months p.i. and transferred to HSV-1 immune recipients. Groups of recipient immune mice received either DLN or CLN cells from thymectomized (Tx) or sham-Tx donors and then challenged with HSV-1. The results are shown in Table 5. Both the DLN and CLN cells from the Tx mice were very efficient at suppressing DTH, whereas only the DLN cells from the sham-Tx mice were effective. Both these groups of mice were still suppressed when tested again 7 days later, indicating that the transfer of suppressor B cells was not a transient phenomenon.
Table 5. Effect of adult thymectomy on the transfer of suppressor cells*

<table>
<thead>
<tr>
<th></th>
<th>Increased ear thickness† (mm x 10⁻³)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>24 h</td>
</tr>
<tr>
<td>Control (no cells)</td>
<td>31.5 ± 3</td>
</tr>
<tr>
<td>Sham-Tx DLN cells</td>
<td>15.9 ± 4†</td>
</tr>
<tr>
<td>Sham-Tx CLN cells</td>
<td>24.5 ± 3</td>
</tr>
<tr>
<td>Tx DLN cells</td>
<td>13.0 ± 2</td>
</tr>
<tr>
<td>Tx CLN cells</td>
<td>13.0 ± 1</td>
</tr>
<tr>
<td></td>
<td>48 h</td>
</tr>
<tr>
<td>Control (no cells)</td>
<td>27.0 ± 2</td>
</tr>
<tr>
<td>Sham-Tx DLN cells</td>
<td>13.0 ± 3</td>
</tr>
<tr>
<td>Sham-Tx CLN cells</td>
<td>22.0 ± 7</td>
</tr>
<tr>
<td>Tx DLN cells</td>
<td>13.0 ± 2</td>
</tr>
<tr>
<td>Tx CLN cells</td>
<td>15.0 ± 5</td>
</tr>
</tbody>
</table>

* Mice were thymectomized 1 month after infection with HSV-1 and left for a further 2 months before transferring 2 x 10⁷ DLN cells/recipient.
† Mean ± S.D. of three mice/group.
‡ Numbers in bold type indicate significant differences when compared to control values (P < 0.001).

DISCUSSION

The control of DTH by specifically sensitized T lymphocytes has been described for several immune systems (Zembala & Asherson, 1973; Bach et al. 1978). In these particular systems the suppressor cells are acting at the sensitization phase, i.e. by competing with or suppressing the precursors or helpers of DTH; the suppressors are usually observed 7 days after sensitization, but are absent by day 14. In contrast to these models we have observed a thy-1-negative, Ig-positive (B cell) population which is active in the DLN from 14 days and up to 9 months following infection with HSV-1. The assay also differs from those previously described in that the suppression is acting against a background of pre-sensitized DTH cells. Another major difference is in the type of antigen used: HSV-1 persists in the host in a latent form, residing in the dorsal root ganglia proximal to the site of the original infection. Consequently, periodic reactivation of infectious virus might occur (Hill et al. 1975) producing a state in which sensitized lymphocytes are re-stimulated to varying degrees.

HSV-1-sensitized B cells have been observed in humans (Rasmussen & Merigan, 1978) and rabbits (Kapoor, 1976) using a stimulation assay. Furthermore, a C 3-positive, Ig-negative lymphocyte population has been described in the rabbit, which suppresses lymphocyte stimulation by HSV-1 (Kapoor & Nash, 1979). The mechanism by which the suppressor B cell operates is unclear, but intervention of anti-HSV-1 antibody appears unlikely since a mouse anti-HSV-1 serum failed to suppress a DTH reaction. Nor does the production of anti-idiotypic activity seem likely, though this is a possibility, since even within the inbred Balb/c strain there is no reason to expect so total a restriction of idiotype specificity. It is more likely that the B cells compete for the virus with pre-sensitized DTH cells or in some way regulate the function of these cells.

An interesting observation was that when immune DLN cells failed to transfer suppression, their pretreatment with anti-thy-1 +c' or removal of Ig-negative cells did allow transfer of suppression. Under these conditions, T cells may interfere with the function of B suppressor cells, i.e. function as suppressors of suppressors. This is consistent with the finding that B cell suppressors are also enhanced following adult thymectomy, indicating that removal of a short-lived T cell population removes a source of regulatory cells which affect B cell suppression. Adult thymectomy also increases B cell suppression in the contralateral lymph node, indicating that such suppressors are present in these nodes, although presumably in reduced numbers, since suppression under normal conditions is virtually ineffective. In fact the contralateral lymph node cells from latently infected mice are more effective at transferring a DTH type response (A. A. Nash, unpublished data).
Suppressor B cells have been described in the guinea-pig which affect the DTH response to ovalbumin in Freund's incomplete adjuvant (Katz et al. 1974). Easmon & Glynn (1979) recently reported a B suppressor against Staphylococcus aureus-induced DTH. It appears likely that with complex antigen systems, such as those met with in infectious diseases, B lymphocytes play an important regulatory role.

What is the significance of this observation for the pathogenesis of HSV infection? First, regulators of inflammatory responses are an essential component of homeostasis if the animal is to survive irreparable immunopathological damage. Second, the balance between suppression and enhancement of the immune system during latency might well be one of the underlying factors involved in virus recurrences. For example, the suppressor system could be involved in inactivating low levels of virus antigens, whereas beyond a certain threshold the suppressor system is inactivated and activation of DTH would take place resulting in the clinical picture of recurrent disease.

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REFERENCES


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