
Key words: HSV-1/interferon (MuIFN-γ)/T cells

T Cell–Macrophage Interactions in the Immune Response to Herpes Simplex Virus: the Significance of Interferon-γ

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(Accepted 21 August 1986)

SUMMARY

The antiviral properties of a herpes simplex virus type 1-specific ‘helper’ T cell clone were investigated. The clone was found to be deficient in interleukin 2 production, although it produced interleukin 3 and interferon-γ upon stimulation with the virus in vitro. Supernatants containing these lymphokines were observed to increase the virocidal activity of macrophages in vitro and furthermore induced these cells to mediate cytotoxic activity against virus-infected target cells. Macrophage activation was linked to the presence of interferon-γ in the clone supernatant. The implications of these results for protection against this virus in vivo are discussed.

It is now well established that cell-mediated immunity plays a central part in the recovery of mice from a primary herpes simplex virus (HSV) infection (Nash et al., 1985; Wildy & Gell, 1985). Using the mouse as a model to study cutaneous HSV infection, an important role was attributed to T cells expressing the Lyt1+ 2− membrane marker in recovery from the infection (Nash & Gell, 1983). Such cells were present in the lymph node draining the site of inoculation of virus and were capable of transferring anti-HSV immunity to syngeneic recipients. In order to investigate further the role of T cells in immunity to HSV, a number of specific Lyt1+ 2− (L3T4-positive) mouse T cell lines were established, from which several T cell clones were derived. One particular clone, D7.1, was shown to inhibit the growth of virus in the skin of syngeneic mice and to protect the mice from an otherwise lethal intraperitoneal virus challenge (Leung et al., 1984). Although this clone ‘helps’ B cells in the induction of an antibody response in vivo, antibody did not appear to correlate with the protection seen in these animals. In addition, factors derived from this clone were able to activate macrophages to kill tumour cell lines in vitro (Leung et al., 1984). In view of this observation, we decided to investigate the interaction between this T cell clone and macrophages as a possible mechanism of anti-HSV immunity.

The derivation and maintenance of clone D7.1 in culture has been described previously (Leung et al., 1984). Briefly, the clone was maintained in RPMI 1640 medium supplemented with 10% foetal calf serum (Flow Laboratories), 10% (v/v) interleukin 2 (IL-2)-rich supernatant [obtained from cultures of MLA144 tumour T cell lines (Rabin et al., 1981)] and a source of infected syngeneic feeder cells [BALB/c spleen cells, X-irradiated and infected with 2 p.f.u. of u.v.-inactivated HSV type 1 (strain SC16)]. The cultures were maintained at 37 °C in a humidified atmosphere of 5% CO2 in air.

To study the production of lymphokines by clone D7.1, a standard procedure was used. Clone D7.1 (8 × 10^5 per culture) was incubated with either feeder cells (10^6 per culture) containing no virus, feeder cells infected with inactivated SC16 or the latter plus 5 μg/ml of a rat monoclonal antibody specific for the L3T4 structure on helper T cells [YTS-191.1 (Cobbold et al., 1984)]. In all cases supernatants were collected after 24 h of culture and tested for the presence of IL-2,
Table 1. Lymphokine production by clone D7.1

<table>
<thead>
<tr>
<th>Supernatant obtained from*</th>
<th>IL-2†</th>
<th>IL-3‡</th>
<th>IFN-γ§</th>
</tr>
</thead>
<tbody>
<tr>
<td>D7.1 alone</td>
<td>&lt;1</td>
<td>&lt;1</td>
<td>&lt;1</td>
</tr>
<tr>
<td>D7.1 + APC</td>
<td>&lt;1</td>
<td>2.8</td>
<td>1.31</td>
</tr>
<tr>
<td>D7.1 + APC + virus</td>
<td>&lt;1</td>
<td>43.0</td>
<td>3.73</td>
</tr>
<tr>
<td>D7.1 + APC + virus + YTS-191.1</td>
<td>&lt;1</td>
<td>&lt;1</td>
<td>1.50</td>
</tr>
</tbody>
</table>

* Eight × 10⁵ D7.1 cells were incubated alone, or with 10⁷ X-irradiated spleen cells as a source of antigen-presenting cells (APC) alone, or with APCs incubated with 2 p.f.u./cell of SC16. YTS-191.1 (anti-L3T4) was added at a final concentration of 5 μg/ml. The cells were incubated for 24 h and supernatants harvested. Estimates of lymphokine activity were carried out in three separate experiments. A representative example is shown in the Table.
† IL-2 results were determined as a percentage of a standard IL-2 preparation obtained from concanavalin A-stimulated mouse spleen cells.
‡ IL-3 results were calibrated as a percentage of a WEHI supernatant (rich in IL-3) taken at a fixed dilution.
§ IFN activity was calculated as described by Atkins et al. (1974). Results are expressed as log₁₀ units.

interleukin 3 (IL-3) and interferon (IFN). The assay for IL-2 was as described by Gillis et al. (1978) using the IL-2-dependent T cell line CTDL and measuring the incorporation of [³H]thymidine. Similarly, the assay for IL-3 depended on the survival of the IL-3-dependent cell line FDCP-1 as measured by [³H]thymidine incorporation (Ihle et al., 1982; Dexter et al., 1980). For comparative purposes, a standard source of IL-3 was obtained from the WEHI-3 cell line (myelomonocytic leukaemia) which spontaneously secretes high levels of IL-3; the supernatant was used at various dilutions to construct a standard curve (Lee et al., 1982). For both these biological assays a Skatron multiwell harvester (Flow Laboratories) was used to harvest the cells from 96-well flat-bottomed plates (Falcon). To assay for IFN-γ the method of Atkins et al. (1974) was used; this depends upon the inhibition of RNA synthesis of Semliki Forest virus (SFV) by IFN. BALB/c mouse embryo fibroblasts were incubated with the clone supernatants for 24 h. In some experiments rabbit anti-mouse IFN-γ or anti-IFN-α/β (a gift from Dr A. G. Morris, University of Warwick, Coventry, U.K.) was added at a 1/100 dilution. The supernatant was then removed and the fibroblasts were infected with approximately 500 p.f.u./cell of SFV in medium containing 5 μg/ml actinomycin D (Sigma) to inhibit RNA synthesis. After 1 h, each well received 1 μCi [³H]uridine (48.7 Ci/mmol, Amersham) and 5 h later the cells were lightly trypsinized and harvested with an automatic cell harvester.

The results of these various assays are shown in Table 1. Under the conditions tested IL-2 was not detected in any of the supernatants obtained from D7.1. The clone was also treated with concanavalin A over a concentration range of 0.1 to 10 μg/ml, and again no IL-2 production was observed (data not shown). Consequently, within the limitations of the biological assay used, we conclude that D7.1 is either a non-producer or a poor producer of IL-2. The absence of IL-2 production in so-called helper T cell clones has been observed previously (e.g. Prystowsky et al., 1982). In contrast to this observation, both IL-3 and IFN were induced when the clone was incubated with feeder cells plus u.v.-inactivated virus. Furthermore, the activation of the clone to produce these lymphokines was blocked by anti-L3T4 antibodies, supporting the view that L3T4 is an essential part of the T cell receptor complex (Swain et al., 1984).

The type of IFN produced by D7.1 was investigated by incubating IFN-containing supernatants with a 1/100 dilution of anti-mouse IFN-α/β or anti-mouse IFN-γ and assaying against SFV. The IFN titre before antibody treatment was 3.36 ± 0.25 log₁₀ units and after treatment with anti-IFN-α/β was 3.5 ± 0.15 log₁₀ units and with anti-IFN-γ was 2.06 ± 0.35 log₁₀ units. These results clearly demonstrate that IFN-γ was produced following the activation of clone D7.1.

In view of the anti-HSV activity of this clone in vivo and the fact that production against the virus did not correlate with antibody activity (Leung et al., 1984), we investigated the interaction of lymphokines derived from clone D7.1 with macrophages. To detect the antiviral activity of these cells two methods were used; one involved the inhibition of virus replication in macrophages, and the other involved macrophage-mediated lysis of virus-infected cells.
Short communication

Fig. 1. Induction of cytotoxic macrophage activity by supernatants derived from clone D7.1. Supernatants were obtained from D7.1 incubated with HSV-infected spleen cells (●), uninfected spleen cells (■) and infected spleen cells plus 5 μg/ml YTS-191.1 (anti-L3T4 monoclonal antibody) (○). The supernatants were diluted 1/100 and added to cells obtained from the peritoneal cavity of BALB/c mice. In one instance the supernatants obtained from D7.1 incubated with virus-infectinf spleen cells were mixed with a rabbit anti-murine IFN-γ serum (1/100 dilution; □). After a 24 h incubation the peritoneal cells were removed and added to virus-infected or uninfected 51Cr-labelled Chang liver cells at different effector:target ratios. The results are expressed as the mean 51Cr release (minus the background counts) of quadruplicate cultures with a deviation from the mean of 10% or less. The background release of 51Cr from infected or uninfected Chang liver cells alone was approximately 15%.

To determine the inhibition of virus growth in macrophages the method described by Bonina et al. (1984) was used. Briefly, macrophages were obtained from the peritoneal cavity of BALB/c mice and dispensed into 24-well plates (Falcon). The adherent cells were retained and incubated with a 1/1000 dilution of D7.1 supernatant for 24 h. Afterwards the cells were infected with SC16 (m.o.i. 1) and incubated for 24 h. The titre of infectious virus was determined at 4 h and 24 h by a standard plaque assay. The yield of virus per 10^6 macrophages at 24 h was 1.09 × 10^3 p.f.u. with supernatants obtained from the clone stimulated by spleen cells plus virus. When untreated macrophages were tested the yield of virus was 1.58 × 10^5 p.f.u., similar to that obtained when the activation of the clones was blocked by anti-L3T4, i.e. 1.47 × 10^5 p.f.u. The yield at 4 h in all groups was approximately 100 p.f.u.

To investigate the ability of the clone supernatants to stimulate cytotoxic activity in macrophages, an assay similar to that described by Chapes & Tompkins (1979) was used. Briefly, peritoneal cells were harvested from normal mice and allowed to adhere to 96-well plastic trays for 2 h. The cells were then incubated with supernatants diluted 1/100 from D7.1. After 24 h the adherent layer (containing 90% macrophages as determined by uptake of nitro blue tetrazolium-coated zymosan particles) was overlaid with 10^4 uninfected or infected (20 p.f.u./cell of SC16) 51Cr-labelled Chang liver cells for 16 h at 37 °C. Half the supernatant was then removed to determine the release of 51Cr by damaged target cells. As shown in Fig. 1, only supernatant from D7.1 activated by feeder cells plus virus produced cell killing. This activity was blocked by anti-L3T4 and anti-IFN-γ, suggesting that macrophage killing was elicited by this IFN.

The activation of macrophages to a heightened virocidal or cytocidal state by T cell products such as macrophage-activating factor is considered to be an important effector arm of the cell-mediated immune response. Two broad mechanisms of macrophage anti-microbial activity have been described: intrinsic activity in which virus replication within the macrophage is
aborted, and extrinsic in which virus replication in other permissive cells is inhibited (Morahan et al., 1980). Previously, Bonina et al. (1984) had reported that arginase activity could be stimulated in macrophages by supernatants from clone D7.1, but the nature of the activating lymphokine in these experiments was not determined. However, it was considered that arginase activity alone was insufficient to restrict virus replication in vivo. A feature of the cutaneous infection with HSV is the increased mononuclear cell infiltration seen at times prior to the elimination of infective virus. By analogy with other infections macrophages predominate at such sites. Consequently, the likelihood that such cells are participating in the antiviral response is high and, as shown in the experiments described herein, activation of macrophages by T cell factors leads to a greatly augmented anti-HSV state. It is possible that macrophage killing of infected cells is an efficient method in vivo. Although a high effector to target ratio was required for killing to be observed, such a situation could be achieved in vivo, particularly where delayed hypersensitivity responses are involved.

In conclusion, we suggest that T cells analogous to D7.1 could exert an anti-HSV effect in the skin by releasing IFN-γ, which interacts with and arms infiltrating macrophages. These cells suppress virus growth either by killing infected cells or by other means, such as by secreting antiviral proteins, e.g. arginase (Wildy et al., 1982) and IFN.

The authors wish to thank Mr C. Bland for expert technical assistance, and Mrs S. Dell for secretarial assistance. This work was supported by a grant from the Medical Research Council of Great Britain, and grant no. 850041704 from the University of Messina.

REFERENCES


(Received 20 May 1986)