Effect of Macrophage Activation on Resistance of Mouse Peritoneal Macrophages to Infection with Herpes Simplex Virus Types 1 and 2

By MARY F. SIT, DANIEL J. TENNEY, JAY L. ROTHSTEIN and PAGE S. MORAHAN

1Department of Microbiology and Immunology, The Medical College of Pennsylvania, 3300 Henry Avenue, Philadelphia, Pennsylvania 19129 and 2Department of Pathology, University of Chicago, 5841 South Maryland Avenue, Chicago, Illinois 60637, U.S.A.

(Accepted 26 April 1988)

SUMMARY

To define the effect of heterogeneity of murine peritoneal macrophages (Mφ) on intrinsic resistance to herpes simplex virus (HSV) infection, several Mφ populations were characterized for their response to infection with HSV type 1 (HSV-1) and HSV-2. Steady-state resident Mφ (Res Mφ) were compared in parallel with Mφ activated with Corynebacterium parvum (now designated Propionibacterium acnes) (CP Mφ) and thioglycollate-elicited inflammatory Mφ (TG Mφ). Res Mφ were completely non-permissive for productive virus infection and showed no c.p.e. The intrinsic resistance of CP Mφ to HSV infection was similar to that of Res Mφ, in that the infection was non-productive for infectious virus, but CP Mφ showed marked c.p.e. TG Mφ showed semi-permissiveness, with virus yields at least 10-fold higher than those in Res Mφ and CP Mφ, and marked c.p.e. The three distinct intrinsic response patterns were maintained regardless of whether Mφ were derived from CD-1 or B6C3F1 mice, or whether the infecting virus was HSV-1 or HSV-2. To define the level at which Mφ restrict HSV replication, immunofluorescence assays for viral antigens and hybridization analyses for viral DNA were performed. All Mφ populations showed immediate early and early virus polypeptides. Res Mφ and CP Mφ showed no viral DNA replication, but TG Mφ showed moderate levels of viral DNA synthesis that paralleled the infectious virus titres produced. Investigation of the mechanism for the heterogeneous intrinsic antiviral response among the Mφ revealed that interferon was not involved, because antiserum to mouse α/β interferon did not alter the intrinsic resistance patterns. Induction of c.p.e. in Mφ required live, replication-competent HSV. The involvement of tumour necrosis factor (TNF) in c.p.e. was found to be unlikely; no significant amounts of TNF were detected in the culture medium of the Mφ, and inclusion of anti-TNF antibody did not inhibit c.p.e.

INTRODUCTION

Macrophages (Mφ) play a highly significant role in non-specific resistance to virus infection, due in part to non-permissiveness of Mφ for replication of many viruses (intrinsic resistance), and inhibition of virus replication in infected permissive cells (extrinsic resistance) (for reviews, see Morahan & Murasko, 1988; Morahan, 1984; Morahan et al., 1985). Mφ, however, display considerable heterogeneity in immune functions including anti-tumour activity, antimicrobial activity, and antigen presentation (Brautigam et al., 1979; Gendelman et al., 1986; Lopez & Dudas, 1979; Morahan et al., 1988; Tenney & Morahan, 1987). This heterogeneity may be a reflection of the state of maturation, differentiation, or activation of the Mφ, or it may be a result of the influence of the local microenvironment of the cell in vivo (reviewed by Morahan et al., 1988).
How Mφ heterogeneity affects resistance to virus infections is not yet well defined. We have previously shown that Mφ from untreated animals, or animals treated with various immunomodulators or eliciting agents, differ in phenotypic and functional properties such as extrinsic antiviral activity (Dempsey et al., 1988; Morahan et al., 1977a, 1982; Morse & Morahan, 1981). There is also heterogeneity in the ability of Mφ to transfer antiviral protection in vivo (Breinig et al., 1978; Schuller & Morahan, 1977), and in intrinsic antiviral resistance (reviewed in Morahan et al., 1988; Gendelman et al., 1986). The resistance of freshly isolated mouse resident peritoneal Mφ (Res Mφ) for infection in vitro by herpes simplex virus (HSV) has long been recognized (Johnson, 1964; Stevens & Cook, 1971), but the molecular mechanisms by which Res Mφ prevent infectious virus from being produced have yet to be defined (Leary et al., 1985). The aim of this study was to determine whether peritoneal Mφ heterogeneity defined by other criteria (Morahan et al., 1988) is also reflected in the intrinsic resistance to HSV, by comparing in parallel the intrinsic antiviral activity of resident, elicited and activated Mφ. We found that peritoneal Mφ heterogeneity is maintained with respect to intrinsic resistance to HSV. We show for the first time that the tumoricidal Corynebacterium parvum (now designated Propionibacterium acnes)-activated Mφ (CP Mφ) population expresses a unique pattern of intrinsic resistance to infection with HSV that has potential implications for Mφ immunotherapy against virus infections. Moreover, only the CP Mφ express both intrinsic and extrinsic antiviral activity to HSV; these activities might be related to the ability of CP treatment to protect mice against HSV infection (Kirchner et al., 1977; Morahan et al., 1977b).

**METHODS**

**Mice.** Barrier-raised female CD-1 (Charles River Breeding Laboratories, Kingston, N.Y., U.S.A.) or B6C3F1 (Ace Animal Laboratories, Boyertown, Pa., U.S.A.) mice were received in filter crates and housed in sterile microisolator cages. The sera of random mice were checked upon arrival and throughout the course of experimentation for seroconversion to common inapparent infections, such as Sendai virus and mouse hepatitis virus, that might alter the response to infection with other viruses and the activation state of the peritoneal Mφ (Dempsey et al., 1986).

**Viruses.** Pools of HSV type 1 (HSV-1) KOS strain and HSV-2 MS strain were prepared in primary rabbit kidney cells (Flow Laboratories). Virus yields were determined by assaying for p.f.u./ml on Vero cells as previously described (Leary et al., 1985). Virus was u.v.-inactivated by irradiation on a gently rotating platform with 5-69 mJ/cm²/s of u.v. radiation for 30 min using a GE number G15T8 lamp. No live virus was detectable after u.v. treatment.

**Reagents.** Brewer thioglycollate broth (TG) (Difco) was prepared as a 10% solution in distilled water, and aged at least 2 weeks prior to use as an Mφ-eliciting agent. Mice were injected intraperitoneally (i.p.) with 0.5 ml TG, 5 days prior to harvesting peritoneal exudate cells (PEC). CP was obtained from Burroughs–Wellcome (Research Triangle Park, N.C., U.S.A.), and injected i.p. (35 mg/kg) 7 days prior to harvesting PEC. Anti-interferon α/β serum (anti-IFN-α/β), prepared in sheep immunized with purified mouse IFN-α/β (Dalton & Pauker, 1981; Pauker et al., 1975), was received courtesy of Dr Barbara Dalton and was titrated by Dr Donna Murasko. The antibody was administered either i.p. in vitro [10⁵ neutralizing units (U)/mouse] 4 days prior to harvesting PEC, to inhibit endogenous IFN-mediated antiviral activity in Mφ (Belardelli et al., 1984; Gresser et al., 1984), or in vitro (10¹ U/ml), 2 h after plating cells. Sheep anti-human IFN-β serum, similarly prepared and injected, was used as a control for any possible serum reaction (no cross-reactivity with mouse IFN). Recombinant human tumour necrosis factor alpha (rHuTNF-α) which is effective in mice and a polyclonal rabbit antiserum that neutralizes rMuTNF-α (anti-TNF) were received courtesy of Genentech (South San Francisco, Ca., U.S.A.). TNF biological activity, assayed by cytotoxic activity against mouse fibrosarcoma cells, was performed as previously described (Urban et al., 1986).

**Mφ culture and infection.** Mφ were cultured at 37 °C in 5% CO₂ in Eagle’s minimal essential medium with Earle’s salts (Sigma) supplemented with 2 mM-L-glutamine, 50 μg/ml gentamicin and 10% heat-inactivated foetal calf serum (Flow Laboratories) for complete medium or 2% serum for maintenance medium. PEC were obtained from mice by peritoneal lavage with 5 ml Dulbecco’s phosphate-buffered saline (PBS) plus 2 units/ml of preservative-free heparin (LymphoMed, Melrose Park, Ill., U.S.A.). PEC were counted, smears were prepared and stained for differentials and cells were resuspended in complete growth medium, plated and Mφ allowed to adhere for 2 h. Non-adherent cells were removed by washing, and the Mφ monolayer was incubated overnight. Mφ and parallel 24 h Vero cell cultures were then washed and nuclei obtained by Pronase–cetrime treatment were counted to determine cell number (Leary et al., 1985). Cultures were infected at an m.o.i. ranging from 2 to 9 p.f.u./cell; there was no significant difference in virus yields or c.p.e. within this range. After 1 h, non-adsorbed
Macrophage intrinsic resistance to HSV

2001

virus was removed by washing twice with PBS and cell cultures were incubated further in maintenance medium. Total yields of virus (cell-associated and supernatant) were determined at specified intervals by plaque titration. The c.p.e. was noted and scored on a scale of 0 (no effect) to 4+ (100% involvement), with +/− indicating less than 5% involvement. For some experiments, c.p.e. was documented by photomicroscopy, using a Leitz inverted microscope with a Nikon HFXII camera and Kodak Panatomic-X ASA 32 film. Attempts were made to quantify the degree of c.p.e. by staining cells with crystal violet, extracting the dye and measuring the optical density, as we have measured Mφ anti-tumour activity (Ackermann et al., 1986). However, because Mφ remain attached although obviously cytotoxic, the c.p.e. was best quantified by traditional microscopic morphological evaluation.

DNA blot assays. Cell preparations were treated with proteinase K (100 μg/ml) and 0.5% SDS for 1 to 2 h and extracted with phenol–chloroform (1:1), followed by chloroform alone. For some experiments, DNA was purified from infected whole cells as previously described (Leary et al., 1985; Maniatis et al., 1982), or from infected cell nuclei (Bell et al., 1981). To degrade RNA and denature DNA, 3.0 M-NaOH was added and the mixture incubated for 30 min at 60 to 70 °C, and neutralized by adding 2 M-ammonium acetate. The DNA preparations were blotted on nitrocellulose filters (Schleicher & Schuell) (Maniatis et al., 1982) and the filters were dried and baked. Plasmids containing EcoRI restriction fragments of the HSV-1 genome (kindly provided by R. Sandri-Goldin; Goldin et al., 1981) were used as probes. DNA probes represented various regions of the HSV-1 genome including EcoRI fragments A (approx. 22 kb) and D (approx. 16 kb) from the unique long portion, fragment H (approx. 15 kb) from the unique short portion, and fragments JK (approx. 18 kb) and EK (approx. 21 kb) from the joint region between the unique and reiterated terminal regions. Several probes were used to ensure that the entire genome was present and replicated. Representative experiments are shown in Results. Plasmid probes were labelled with [32p]dCTP (sp. act. 800 Ci/mmol) by nick translation using commercially available kits (Rigby et al., 1977; Bethesda Research Laboratories or Worthington) achieving sp. act. of approximately 10^7 to 10^8 c.p.m./μg DNA. Prehybridization and hybridization conditions were as previously reported (Maniatis et al., 1982). Following high stringency washes, filters were exposed to X-ray film with intensifying screens.

Immunofluorescence. Cells were grown on 12 mm circular coverslips and, at various times following infection with HSV-1, were fixed in acetone for 15 to 20 min and air-dried. Immunofluorescence for HSV-1 antigens was assayed either directly, using fluorescein-labelled monoclonal antibody (MAb) to an HSV-1-specific, Mr 155K cytoplasmic protein that appears to be an early protein (Kallestad Laboratories, Austin, Tx., U.S.A.) or indirectly, using a MAb to the HSV-1 immediate early protein ICP4 (Ackermann et al., 1984; courtesy of L. Pereira) followed by fluorescein-labelled rabbit anti-mouse IgG (Miles Laboratories) as previously described (Tenney & Morahan, 1987). Some experimental results were documented by photomicroscopy using a Zeiss Universal microscope equipped for fluorescein isothiocyanate fluorescence and a Nikon HFXII camera with Kodacolor VR400 print film.

RESULTS

Mφ heterogeneity with respect to HSV-1 and HSV-2 yields

Consistent differences in resistance to HSV-1 infection were exhibited by various peritoneal Mφ populations (both CD-1 and B6C3F1 mice) in total yield of infectious virus (p.f.u.) and in virus-induced c.p.e. (Table 1, Fig. 1 and 2). The mouse prototype tissue Mφ population, Res Mφ, exhibited a marked reduction of input virus, to levels below that expected from thermal inactivation alone (Leary et al., 1985). Little or no virus c.p.e. was observed beyond transient

Table 1. HSV-1 production in Mφ from CD-1 and B6C3F1 mice

<table>
<thead>
<tr>
<th>Cell source</th>
<th>Cell type</th>
<th>Geometric mean maximum p.f.u./cell</th>
<th>Range of c.p.e. at 24 h</th>
<th>No. of experiments</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD-1 mouse</td>
<td>Res Mφ</td>
<td>0.004</td>
<td>0 to 1+</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td>TG Mφ</td>
<td>0.199*</td>
<td>2+ to 4+</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>CP Mφ</td>
<td>0.002</td>
<td>1+ to 4+</td>
<td>4</td>
</tr>
<tr>
<td>B6C3F1 mouse</td>
<td>Res Mφ</td>
<td>0.001</td>
<td>0 to 1+</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>TG Mφ</td>
<td>0.047*</td>
<td>2+ to 3+</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>CP Mφ</td>
<td>0.006</td>
<td>2+ to 4+</td>
<td>4</td>
</tr>
<tr>
<td>African green monkey</td>
<td>Vero</td>
<td>200</td>
<td>2+ to 4+</td>
<td>13</td>
</tr>
</tbody>
</table>

* P < 0.05 when the geometric means were compared with the yield in Res Mφ by Student’s t-test.
Fig. 1. Differences in c.p.e. caused by HSV-1 in various peritoneal Mφ populations. B6C3F1 Res Mφ (b, f), TG Mφ (c, g) and CP Mφ (d, h), as well as Vero cells (a, e), were infected with HSV-1 at an m.o.i. of approximately 3. The control is shown (a to d) and the c.p.e. is shown at 24 h p.i. (e to h) with the following results: Vero 4+, Res Mφ 0, TG Mφ 3+ and CP Mφ 4+. At 24 h p.i., the p.f.u./cell yield was 401.1 for Vero and ranged from 0.002 to 0.02 for Res Mφ, TG Mφ and CP Mφ. Bar marker represents 50 μm.

granulation and an increased spreading that was sometimes observed 24 to 48 h post-infection (p.i.). Activated CP Mφ were similar to Res Mφ in that input virus infectivity was markedly reduced, but CP Mφ showed a significantly greater c.p.e., becoming rounded and pyknotic within 24 h p.i. (Fig. 1). In thioglycollate-elicited inflammatory Mφ (TG Mφ) the mean yield per cell of infectious virus was significantly greater than the yield from Res Mφ or CP Mφ (Table 1). Although the HSV yields varied between experiments, in each one the yield from TG Mφ was at least 10-fold greater than from Res Mφ. TG Mφ also exhibited significant c.p.e., varying from 2+ to 4+ at 24 h p.i. (Fig. 1). Similar to CP Mφ, the extent of c.p.e. did not correlate with the amount of virus produced. Vero cells also varied in c.p.e. from 2+ to 4+ at 24 h p.i., with no correlation in the amount of virus produced. Infection of B6C3F1 Res Mφ, TG Mφ and CP Mφ with HSV-2 produced similar results to those described for infection with HSV-1.
Macrophage intrinsic resistance to HSV

However Mφ infected with HSV-2 exhibited a slightly greater c.p.e. than parallel cultures infected with HSV-1 (Fig. 2), probably because of the more rapid shut-off of host polypeptide synthesis caused by HSV-2 (Schek & Bachenheimer, 1985; Sydiskis & Roizman, 1967).

Because peritoneal cell populations, especially those induced by inflammatory agents, can be contaminated with fibroblasts that are permissive for HSV infection, we took several precautions to rule out any significant fibroblast contamination. Mφ were infected within 24 h after harvest to reduce the possible effects of fibroblast overgrowth during longer culture in vitro. In some experiments, adherent TG Mφ were treated before infection with 0.25% trypsin alone or with 0.05% trypsin and 0.025% EDTA to remove fibroblasts without affecting adherent Mφ (Tushinski et al., 1982). There were no major differences in the c.p.e. or production of infectious virus in the trypsinized TG Mφ (2+ to 3+ c.p.e. and yield of 0.02 to 0.04 p.f.u./cell) and control TG Mφ (2+ to 3+ c.p.e. and yield of 0.09 p.f.u./cell). Experiments were also performed in which treated and untreated TG Mφ were cultured for 6 to 7 days before infection. If there were significant (1%) contamination of the TG Mφ with fibroblasts, the latter should replicate to approximately 4 to 8% of total cells over the 7 days so that differences in virus yields should be amplified. However, no major differences were apparent either in c.p.e. or virus yields when such TG Mφ were infected with HSV.

Viral DNA and protein synthesis

In CD-1 or B6C3F1 (data for the latter not shown) Res Mφ, input HSV-1 DNA was present at 2 h p.i., but there was no increase by 24 h p.i. (Fig. 3a to d). CP Mφ of either mouse strain were also similar to the Res Mφ (Fig. 3a to d). TG Mφ exhibited a variable pattern of DNA synthesis, related to the production of infectious virus. When a moderate increase in p.f.u. was observed over the course of infection, there was an increase in viral DNA synthesis (Fig. 3e to h). When input infectivity decreased slightly over time, viral DNA replication was not detectable (Fig. 3a to d). Experiments in which DNA was extracted from HSV-1-infected Res Mφ and Vero cell nuclei (as opposed to whole cell DNA extraction) showed that input viral DNA was able to enter the Res Mφ nucleus (Fig. 4). Therefore, experiments were undertaken to determine whether HSV-1 initiated any early polypeptide synthesis in Mφ.
Fig. 3. (a to d) Kinetics of synthesis of HSV-1 DNA in peritoneal Mφ, CD-1 Res Mφ (column 1), TG Mφ (column 2) and CP Mφ (column 3) as well as Vero cells (column 4) were infected with HSV-1 KOS. DNA from uninfected controls (a) and infected cells (b, 2 h; c, 4 h; d, 24 h) was blotted at 5 x 10⁴ cells/well. A representative experiment, using pBR325 containing the 22 kb EcoRI genomic restriction fragment A from HSV-1 KOS as the probe is shown. The yield of HSV-1 was < 0.1 p.f.u./cell for all Mφ. (e to h) Infected CD-1 and Vero cell DNA was purified and blotted at 1 μg (e, control; f, 2 h; g, 6 h; h, 24 h). A representative experiment using pBR325 containing the 21 kb EcoRI genomic restriction fragment EK from HSV-1 KOS as the probe is shown. TG Mφ yielded 1.1 p.f.u./cell at 24 h. Column 5, TG Mφ; column 6, Res Mφ; column 7, Vero cells.

Fig. 4
Macrophage intrinsic resistance to HSV

Table 2. Kinetics of HSV-1 antigen production

<table>
<thead>
<tr>
<th>Cell type</th>
<th>Antigen</th>
<th>1 h</th>
<th>3 h</th>
<th>6 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vero</td>
<td>ICP4</td>
<td>0 (1)*</td>
<td>44 (3)</td>
<td>73 (3)</td>
</tr>
<tr>
<td></td>
<td>155K</td>
<td>0 (1)</td>
<td>15 (3)</td>
<td>55 (3)</td>
</tr>
<tr>
<td>Res MΦ</td>
<td>ICP4</td>
<td>0 (3)</td>
<td>8 (4)</td>
<td>12 (4)</td>
</tr>
<tr>
<td></td>
<td>155K</td>
<td>3 (3)</td>
<td>15 (5)</td>
<td>23 (4)</td>
</tr>
<tr>
<td>TG MΦ</td>
<td>ICP4</td>
<td>0 (3)</td>
<td>6 (4)</td>
<td>16 (3)</td>
</tr>
<tr>
<td></td>
<td>155K</td>
<td>1 (3)</td>
<td>6 (5)</td>
<td>19 (4)</td>
</tr>
<tr>
<td>CP MΦ</td>
<td>ICP4</td>
<td>0 (1)</td>
<td>7 (2)</td>
<td>10 (2)</td>
</tr>
<tr>
<td></td>
<td>155K</td>
<td>2 (3)</td>
<td>9 (4)</td>
<td>12 (2)</td>
</tr>
</tbody>
</table>

* Average % of antigen-positive cells, with the number of experiments shown in parentheses.

Immunofluorescence assays indicated that in all three MΦ populations, synthesis of the immediate early ICP4 protein occurred, although not to the extent observed in the fully permissive Vero cells (Table 2, Fig. 5). All three MΦ types also showed synthesis of the 155K cytoplasmic protein at levels about half that of positive Vero cells (Table 2).

Investigation of possible mechanisms involved in the heterogeneity in intrinsic resistance of MΦ to HSV

Anti-mouse IFN-α/β serum was administered i.p. to mice 4 days prior to harvesting PEC, in order to determine whether neutralization of IFN-α/β in vivo might reduce an endogenous antiviral state in peritoneal MΦ and increase permissiveness for HSV-1 replication in vitro. Anti-IFN-α/β was also added in vitro to MΦ cultures 2 h after plating, so that any IFN-α/β produced under culture conditions might be neutralized. No increase in virus titre was observed in TG MΦ following infection with HSV-1 when anti-IFN-α/β was administered in vivo, in vitro or both, and there was no difference in the amount of c.p.e. produced (Table 3). Likewise, no significant increase in virus titre was observed in Res MΦ following HSV-1 infection when anti-IFN-α/β was administered in vivo, while a slight increase in titre and c.p.e. was noted in the group with anti-IFN-α/β in vitro and in the combined in vivo/in vitro anti-IFN-α/β treatment.

Mechanisms that might be involved in the cytotoxicity that TG MΦ and CP MΦ exhibit after infection with HSV were also investigated. Live virus was required for cytotoxicity to occur; no cytotoxicity was produced by infection with a comparable m.o.i. of u.v.-inactivated virus (data not shown).

Sensitivity to the cytotoxic effects of TNF has recently been reported to be increased in some virus-infected cells (Koff & Fann, 1986; Wong & Goeddel, 1986). We hypothesized that HSV infection might induce TG MΦ or CP MΦ to secrete TNF, and that secreted TNF might be selectively toxic for HSV-infected TG MΦ or CP MΦ. We investigated this possibility in several ways. (i) The amount of TNF present in MΦ cultures was measured (Urban et al., 1986). There were no significant amounts of TNF in any of the supernatant fluids taken from uninfected or HSV-1-infected (24 h p.i.) Vero cells or Res MΦ, TG MΦ or CP MΦ. None of the supernatant fluids showed any cytotoxicity beyond a 1:4 dilution, after any live HSV had been inactivated by treatment with antibody or with u.v. radiation. (ii) The ability of exogenously added rHuTNF-α
Fig. 5. Synthesis of the HSV-1 immediate early protein ICP4 in B6C3F1 Res Mφ (c), TG Mφ (a), CP Mφ (b) and Vero cells (d). Mφ and Vero cells were mock-infected or infected with HSV-1, cells were fixed for fluorescence at 6 h, and the presence of antigen was detected by indirect immunofluorescence using a MAb to ICP4. Only the mock-infected Res Mφ slide is shown (c) because all control preparations were negative. Bar marker represents 100 μm.

to be cytotoxic for uninfected or HSV-1-infected Mφ was assessed by adding 10⁵ units of rHuTNF-α (Kramer & Carver, 1986). No cytotoxicity resulted in the uninfected Mφ or infected Res Mφ, and no enhanced c.p.e. was observed in the infected TG Mφ and CP Mφ. (iii) We also measured the ability of rMuTNF-α-neutralizing antiserum to reverse the cytotoxicity in HSV-infected TG Mφ. TG Mφ were infected with HSV, and immediately treated with 3·2 × 10⁴ U of anti-rMuTNF-α serum. This treatment, however, did not prevent the cytotoxic effect of the virus infection, and did not alter the amount of virus produced. TG Mφ treated with control antiserum showed 4+ c.p.e. and a yield of 0·05 p.f.u./cell, as compared with TG Mφ treated with anti-TNF antiserum which showed 4+ c.p.e. and a yield of 0·07 p.f.u./cell. (iv) The possibility that cytotoxic factors other than TNF might be involved was assessed by measuring
Macrophage intrinsic resistance to HSV

Table 3. Endogenous interferon does not alter permissiveness of Res Mφ and TG Mφ for HSV-1

<table>
<thead>
<tr>
<th>Treatment*</th>
<th>Cell type</th>
<th>Yield at 24 h (p.f.u.)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Per well</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(log10)</td>
</tr>
<tr>
<td>Anti-IFN</td>
<td></td>
<td></td>
</tr>
<tr>
<td>in vivo/in</td>
<td>Vero</td>
<td>6.4 to 8.4</td>
</tr>
<tr>
<td>vitro</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Res Mφ</td>
<td>2.6</td>
</tr>
<tr>
<td></td>
<td>TG Mφ</td>
<td>5.2</td>
</tr>
<tr>
<td>Hu-β</td>
<td>Res Mφ</td>
<td>2.9</td>
</tr>
<tr>
<td>In vivo</td>
<td>TG Mφ</td>
<td>4.9</td>
</tr>
<tr>
<td>Mu-α/β</td>
<td>Res Mφ</td>
<td>2.6</td>
</tr>
<tr>
<td>In vivo</td>
<td>TG Mφ</td>
<td>5.2</td>
</tr>
<tr>
<td>Mu-α/β</td>
<td>Res Mφ</td>
<td>3.8</td>
</tr>
<tr>
<td>In vitro</td>
<td>TG Mφ</td>
<td>4.6</td>
</tr>
<tr>
<td>Mu-α/β</td>
<td>Res Mφ</td>
<td>4.2</td>
</tr>
<tr>
<td>In vivo/in</td>
<td>TG Mφ</td>
<td>4.8</td>
</tr>
<tr>
<td>vitro†</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* CD-1 mice were either untreated or inoculated with TG 5 days before, and then injected i.p. with 10^5 U of anti-mouse IFN-α/β or anti-human IFN-β (control) serum 4 days before harvesting peritoneal cells (in vivo groups). The in vitro groups had no anti-interferon in vivo, but freshly adhered Mφ were treated with anti-IFN serum for 24 h prior to and during HSV-1 infection. The anti-IFN serum treatment was sufficient to neutralize 100000 international units of IFN, an amount far greater than that present in normal animals or uninduced Mφ.

† This group received anti-IFN serum treatment both in vivo and in vitro.

the ability of supernatant fluids from infected Mφ to be cytotoxic for other Mφ. No cytotoxic effects occurred when supernatant fluids from HSV-infected Res Mφ, TG Mφ and CP Mφ were diluted 1:3 and incubated with any of the three types of uninfected Mφ.

**DISCUSSION**

The present data establish that there are consistent patterns among mouse peritoneal Mφ in intrinsic resistance to HSV infection (Morahan et al., 1977b, 1982). Res Mφ, TG Mφ and CP Mφ could be distinguished in their intrinsic resistance, whether they were infected with HSV-1 or HSV-2, or whether the Mφ were derived from the CD-1 outbred or B6C3F1 inbred mouse strain. Thus these Mφ types, resident, inflammatory and activated, can be separated according to their intrinsic antiviral resistance, in addition to previously described surface phenotypes and functional distinctions (Morahan et al., 1977b, 1982). We are now defining the molecular mechanisms involved in these differences in antiviral resistance in order to determine the relationship to Mφ differentiation in either model systems in vitro (Tenney & Morahan, 1987) or in the intact animal.

The unique intrinsic response that we report for the first time for CP Mφ to HSV is intriguing. Activated Mφ also express marked extrinsic antiviral activity in vitro (Morahan et al., 1977b; Morse & Morahan, 1981). In addition, treatment of mice with immunomodulators such as CP produces marked antiviral resistance in vivo (Kirchner et al., 1977; Morahan et al., 1977b), and antiviral and antitumour resistance can be transferred in vivo by administration of activated peritoneal cells (Breinig et al., 1978; Kaplan & Morahan, 1976; Schuller & Morahan, 1977). However, CP Mφ, although resistant to productive infection with HSV in vitro, were very susceptible to the cytotoxic effects of HSV infection. It remains to be determined whether the destruction of HSV-infected CP Mφ plays a role in the antiviral resistance that is observed in vivo, or if the c.p.e. could be an adverse side effect of antiviral immunotherapy with immunomodulators.

The intrinsic resistance pattern that we describe for the prototype inflammatory Mφ population, TG Mφ, is similar to that which we have previously demonstrated for bone marrow-derived Mφ (Leary et al., 1985). These Mφ most closely resemble inflammatory Mφ, and both are probably less differentiated than the steady-state Res Mφ (Dempsey et al., 1988; Van der Meer et al., 1983). The current data with TG Mφ extend the early reports that TG Mφ produce
more infectious HSV than do Res Mφ (reviewed in Morahan, 1984; Morahan et al., 1985). Our results establish that TG Mφ are very susceptible to the c.p.e. of HSV, and emphasize that there is heterogeneous expression of viral antigens and virus replication among the TG Mφ population. The heterogeneity in response to HSV infection may be related to the well-established heterogeneity that is apparent in the TG Mφ population in terms of cell size, morphology, ectoenzyme profile, etc. (Morahan et al., 1982). The mechanisms responsible for the heterogeneous expression of HSV genes in TG Mφ are currently being investigated by in situ hybridization.

The present results with TG Mφ are also reminiscent of the seminal studies of Stevens & Cook (1971). They found that HSV infection of murine Res Mφ that had been cultured for 2 days in calf and rabbit serum (to enhance spreading) resulted in some viral DNA synthesis, declining titres of infectious HSV, and considerable c.p.e. Such cultured Mφ were likely to have either been partially activated in vitro or in vivo by inapparent virus infections in mice that were not recognized 17 years ago (Dempsey et al., 1986).

The mechanism for the selective cytotoxicity of CP Mφ and TG Mφ to HSV infection remains to be completely defined. The hypothesis we tested was that HSV infection would induce inflammatory or activated Mφ to synthesize and secrete soluble cytotoxic factors such as TNF (Gifford & Lohmann-Matthes, 1986; Urban et al., 1986), which could then be selectively toxic for virus-infected Mφ. There have been a few recent reports of the ability of certain viruses to induce TNF (Berent et al., 1986), and for TNF to have antiviral activity or selective toxicity for virus-infected cells (Koff & Fann, 1986; Mestian et al., 1986; Wong & Goeddel, 1986). However, we did not find significant levels of TNF in the supernatant fluids of either uninfected or infected Mφ. Thus, these cells did not produce TNF endogenously, and HSV infection did not induce synthesis of TNF. It is possible that the TNF might have remained membrane-bound and thus not been secreted (Decker et al., 1987). However, when potent neutralizing antiserum to MuTNF-α was added, the cytotoxic effect of HSV on TG Mφ remained. Moreover, we have not been able to transfer cytotoxicity with various supernatant fluids or with recombinant TNF. It is still possible that a very labile soluble factor(s) may be involved. However, we are currently investigating whether certain Mφ may be very sensitive to the disaggregation of polyribosomes and degradation of cellular mRNAs which accompanies HSV infection in permissive cells (Nishioka & Silverstein, 1978; Schek & Bachenheimer, 1985; Sydiskis & Roizman, 1967). This susceptibility may be induced by a very early event in HSV replication which occurs in all the Mφ. Activated Mφ have decreased synthesis of RNA and altered processing of ribosomal RNAs (Varesio et al., 1987), so that additional perturbation of cellular RNA at an early stage in HSV replication, such as transcription of immediate early genes, might be toxic for cells. We are currently performing in situ hybridization to detect such viral mRNA.

The present data emphasize that the mechanism(s) involved in the intrinsic resistance of mouse peritoneal Mφ to HSV-1 or HSV-2 infection differs from those operative against vesicular stomatitis virus (VSV) infection (Belardelli et al., 1984; Gresser et al., 1984; Vogel & Fertsch, 1987). Freshly harvested peritoneal Mφ appear to be resistant to VSV through an antiviral state induced by exposure of Mφ to endogenous IFN in vivo, because treatment of mice with anti-IFN serum in vivo abolished the intrinsic resistance of such Mφ to VSV. In our studies, however, similar treatment in vivo with anti-IFN serum did not alter the degree of intrinsic resistance to HSV-1 that was exhibited by either Res Mφ or TG Mφ. Treatment of Mφ in vitro with anti-IFN serum did increase by 10-fold the titre of HSV in Res Mφ, suggesting that HSV infection might normally induce Res Mφ to produce IFN that is partially responsible for the resistance of Res Mφ to HSV. Others have recently reported that treatment of certain Mφ in vitro with IFN or other antiviral lymphokines produces marked antiviral activity against herpesviruses (Bielefeldt Ohmann et al., 1984; Domke-Optiz et al., 1986; Ellermann-Eriksen et al., 1986; Rose et al., 1986; Straub et al., 1986). Our results emphasize the fact that Mφ have a variety of intrinsic antiviral mechanisms that may be brought to bear on virus infections, and that one common antiviral mechanism is unlikely. The challenge for antiviral immunotherapy with Mφ is how to produce Mφ with the precise antiviral mechanism desired without accompanying adverse effects, such as susceptibility to virus cytotoxicity.
The authors thank Dr Hans Schreiber for the design and assay in the TNF experiments, Dr Mark Cosentino for critical discussions and expert technical assistance, Tracey Bowman and Saifuddin Mama for expert technical assistance, Dr Patricia M. Repik for critical comments, and Liz Newsome and Joyce Smith for preparation of the manuscript. The gifts of monoclonal antibodies from L. Pereira, plasmids from R. Sandri-Goldin, anti-IFN serum from B. Dalton, IFN titres by D. Murasko, recombinant human TNF and rabbit anti-TNF antisera from M. Shepard, and HSV-1 (KOS) stocks from K. Leary are greatly appreciated. This work was supported by Public Health Service grants CA35961, CA19266, CA22677 and CA37156 from the National Cancer Institute, grant IM462 from the American Cancer Society, contract DAMD17-86-C-6117 from the U.S. Army Medical Research Command, and training grant T32 A107090.

REFERENCES


(Received 4 January 1988)