Autocrine secretion of interferon-α/β and tumour necrosis factor-α synergistically activates mouse macrophages after infection with herpes simplex virus type 2

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Introduction

Macrophages (Mφ) are generally considered to play an essential role in resistance to virus infections (Mogensen, 1979; Haller, 1981; Mogensen & Virelizier, 1987). Although both intrinsic (Mogensen, 1979) and extrinsic (Morahan, 1984) antiviral activities of Mφ have been described for a number of virus infections, the basic nature of these antiviral principles and their induction and regulation are not yet elucidated.

During infection of Mφ with herpes simplex virus type 2 (HSV-2) in vivo and in vitro we and others have previously shown that the cells acquire an increased capacity to react with a respiratory burst after membrane triggering (Mogensen et al., 1989; Giridhar et al., 1991). However infection of a macrophage cell line and mononuclear phagocytes with vesicular stomatitis virus or influenza virus for a short period (20 min to 3 h) either did not influence the respiratory burst or reduced it (Rager-Zisman et al., 1982; Abramson et al., 1982). Furthermore, short-term (30 min) infection of polymorphonuclear leukocytes with different viruses resulted in very different reactions of the cells; some viruses enhanced the respiratory burst and some diminished it (Faden et al., 1979). Previously we have shown that the HSV-2-induced priming takes some hours to develop, and that it peaks at about 8 to 12 h, after which it wanes (Mogensen et al., 1989). These data indicate that virus-induced activation of leukocytes is a complicated phenomenon probably depending on factors like cell type, the virus in question, the infectious load and the timing after the infection. The role of the reactive oxygen species in resistance to virus infections is largely unknown, although it has been documented that oxygen radicals produced by polymorphonuclear leukocytes or Mφ-like cell lines can reduce the infectivity of both extracellular and intracellular virus (Jones, 1982; Rager-Zisman et al., 1982), and that chemically induced superoxide has an antiviral effect in HeLa cells, which could be relevant for the antiviral effect of interferon (IFN) (Huang et al., 1992). Furthermore, it has been shown that oxygen radicals are involved in the patho-
genesis of virus infections, promoting the symptomatology (Oda et al., 1989; Ikeda et al., 1992).

Herpes simplex virus type 1 (HSV-1) and HSV-2 infection of resting mouse peritoneal MΦ in vivo and in vitro induces these cells to produce interferon-α/β (IFN-α/β) very early after the infection (Kirchner et al., 1983; Ellermann-Eriksen et al., 1986b; Mogensen & Virelizier, 1987). Furthermore, it is known that IFN-α/β is able to prime MΦ for a respiratory burst (Ito et al., 1985; Ellermann-Eriksen et al., 1989), and we have shown that IFN-α/β plays a role in the priming of MΦ after HSV-2 infection (Mogensen et al., 1989; Ellermann-Eriksen et al., 1989). The present study focuses on the importance of cytokines in the HSV-2-induced activation of MΦ for an increased respiratory burst. It is shown that secretion of IFN-α/β by MΦ in response to HSV-2 infection in an autocrine manner activates MΦ as measured by priming of the cells for a respiratory burst, and that autocrine secretion of tumour necrosis factor-α (TNF-α), also in response to the infection, synergistically enhances the IFN-α/β-induced MΦ activation.

**Methods**

**Mice.** Inbred, specific pathogen-free C57BL/6J-BOM and BALB/cABOM mice were originally obtained from Bornholmgaard Animal Breeding and Research Center and bred locally behind specific pathogen-free barriers. The mice were regularly checked to be free of mouse hepatitis virus, reovirus 3, pneumonia virus of mice, Sendai virus, and minute virus of mice. Mice of each sex were used at the age of 8 to 12 weeks, but for individual experiments only mice born in the same week and of a single strain and sex were used.

**Virus.** The MS strain of HSV-2 used throughout this study has been described previously by Mogensen et al. (1974). A high-titre stock of virus was produced by infecting monolayers of Vero cells (mycoplasma-free) grown in 11 Roux bottles in 100 ml of Eagle’s MEM containing 2% fetal calf serum (FCS), 200 international units (IU) per ml of penicillin and 200 µg/ml of streptomycin, at a m.o.i. of 0.01. At almost complete c.e.p.e. (48 h), the bottles were subjected to two cycles of freezing (−70°C) and thawing, and the supernatant was clarified of cellular debris by centrifugation at 3000 g for 30 min. The virus was pelleted by ultracentrifugation at 45000 g for 1 h, resuspended in RPMI medium (RPMI-1640 medium with 5% FCS, 10 mM-glutamine, 2 mM-HEPES, and antibiotics as above). After sonication at 40 W for 30 s three times the virus preparation was divided into samples and stored at −70°C. The virus stock had an infectivity titre of 108 p.f.u./ml. Just before usage virus was thawed and used as infective preparations. (i) Crude IFN-α/β (IFN-MΦ) was produced by peritoneal MΦ infected for 24 h with Newcastle disease virus (NDV) as previously described (Mogensen et al., 1989). Briefly, peritoneal MΦ from C57BL/6 mice were infected with NDV (strain H 414, the State Serum Institute, Copenhagen, Denmark), allowed to adsorb the virus for 2 h, washed five times, and incubated for 22 h in RPMI medium as above with 10% FCS. After harvesting the supernatant was adjusted to pH 2.0 for 24 h, readjusted to pH 7.4, and stored at −70°C. This MΦ IFN was of the α/β type since it was acid-stable and neutralized by a goat antiserum raised against mouse IFN-α/β (originating from Dr E. DeMaeyer, Orsay, Paris, France), which does not exhibit neutralizing activity against IFN-γ (DeMaeyer & DeMaeyer-Guignard, 1983). (ii) Purified IFN-α (sp. act. 2 x 10⁸ U/mg protein) was purchased from Lee BioMolecular (cat. no. 22061, lot 87077). This IFN preparation is acid-stable, and it is not neutralized by rabbit antibodies to IFN-β with <0.02% cross-reactivity towards mouse IFN-α, thus containing less than 0.5% of the β-type (manufacturer’s information). Recombinant murine TNF-α (sp. act. 4 x 10⁸ U/mg protein) was purchased from Genzyme (cat. no. TNF-M, lot B8120) and had a purity of more than 99% (manufacturer’s information).

**Cytokine assays.** Before assays of cytokine activity were performed the test supernatants (1 ml) were subjected to u.v.-inactivation for 15 min in a 24-well multidish tray (Nunc) 10 cm from a 15 W u.v. source (Philips).

IFN activity in supernatants was measured in a microtitre assay with vesicular stomatitis virus as challenge virus. Briefly, 10⁴ L-929 cells were seeded in twofold dilutions of the test supernatants in the wells of Nunc MicroWell plates, incubated for 20 h, and infected with 500 p.f.u. of vesicular stomatitis virus for 48 h. The cells were fixed in 1% formaldehyde and stained with crystal violet (1 mg/ml). The IFN titre was determined from duplicate rows as the dilution reducing the c.e.p.e. by 50% and compared with a standard IFN-α (Lee BioMolecular, described above, equilibrated with NIH and NIBSC reference preparations) titrated in the central row of each plate. The antiviral principle was identified as IFN-α/β by neutralization with the specific goat antiserum to murine IFN-α/β described above.

**TNF-α activity** was measured in a cytotoxicity assay performed in L-929 cells. Monolayers of cells seeded the day before (5 x 10⁴ cells/well) were overlaid with twofold dilutions of the supernatants and incubated at 38.5°C with actinomycin D (1 µg/ml; Calbiochem, Behring Diagnostics) for 18 h. The plates were fixed and stained as above, and light absorbance at 600 nm was measured in an ImmunoReader (InterMed). TNF titres were assessed as the dilution resulting in 50% cytotoxicity and compared with a standard TNF-α (Genzyme; described above) titrated in the central row of each plate. The cytotoxic
principle was identified as TNF-α by neutralization with the specific antiserum to murine TNF-α described above.

Chemiluminescence assay. The activity of Mφ was assessed by the ability of the cells to generate a respiratory burst as measured by luminol-dependent chemiluminescence after triggering with phorbol 12-myristate 13-acetate (PMA; Sigma) as previously described (Mogen-sen et al., 1989). Briefly, cells in polystyrene cuvettes were transferred from the incubator to a 37 °C water-bath 5 min before the start of the assay and 160 μl of a 0.5 mm solution of 5 amino-2,3-dihydro-1,4-phthalazinedione (luminol; Fluka Chemie) was added, giving a final concentration of luminol of 80 μM. Cells were triggered for respiratory burst by adding 40 μl of a 10 μg/ml solution of PMA, resulting in a final concentration of 0.4 μg/ml (0.65 μM). PMA and luminol were dissolved in DMSO, and further diluted in RPMI medium resulting in a final DMSO concentration of 0.2% in the cultures. The luminol-dependent chemiluminescence was measured in a luminometer (LKB Wallac 1250, LKB Instruments) at each min for the first 5 min and at indicated times for a total of 15 min.

Statistics. Statistical analysis was performed on log_{10} values of the peak chemiluminescence response by Student’s t-test. Synergy was tested on values expressed as [(result from a culture/mean of controls)−1] by Student’s t-test. The distribution of results from double-treated cells was compared with the distribution derived from the addition of the two distributions from single-treated cells [mean = mean1+mean̅ 2, SD = √SD₁²+SD₂², n = (n₁ + n₂)/2].

Results

Effect of infectious and non-infectious HSV-2 on the respiratory burst of peritoneal Mφ and the concomitant production of IFN-α/β and TNF-α

Peritoneal Mφ were infected with 10^5 to 10^7 p.f.u./ml of HSV-2 and the chemiluminescence response was measured 10 h later. A dose-dependent activation of the cells giving an increased capacity for PMA-induced respiratory burst after infection with HSV-2 was seen (Fig. 1a). The activation level for a respiratory burst peaked at an infectious dose of 3.2 × 10^6 p.f.u./ml (m.o.i. of 1.1; P = 0.0007) and declined when the cells were infected with a higher infectious dose. This pattern was seen repeatedly with several stocks of virus. Heat inactivation of the virus resulted in a total inhibition of the activating potential, whereas HSV-2 inactivated by u.v. light still had a priming effect on the peritoneal cells. This effect was of the same magnitude or lower than that of infectious HSV-2, except with the highest dose of u.v.-inactivated virus, where activation of the Mφ was higher than that with infectious virus, as no decline in activation was seen. No residual infectivity was found in the inactivated HSV-2 preparations when titrated in a plaque assay.

Titrations of IFN-α/β produced during the 10 h of infection showed that increasing infectious doses augmented the amount of IFN-α/β produced, and that there was no decline in the production from cells infected with the highest dose of virus. Heat-inactivated HSV-2 did not induce any production of IFN-α/β, whereas u.v.-inactivated HSV-2 resulted in significant production in response to the highest dose of virus (Fig. 1b). When TNF-α in the supernatants from cells after 10 h of

![Fig. 1. Respiratory burst and cytokine production of mouse peritoneal cells subjected to infectious, u.v.-inactivated and heat-inactivated HSV-2. Peritoneal cells were infected with various doses of HSV-2 (●) at an m.o.i. of 0.03 to 3.3, or the corresponding amounts of u.v.-inactivated (▲) or heat-inactivated (○) virus were added, and the cultures were incubated for 10 h. The peak luminol-dependent chemiluminescence response was measured after PMA triggering (a). The supernatants were assayed for IFN-α/β activity (b), and for TNF-α activity (c). Each point represents the mean value from two cultures, except controls (□) which represent four cultures, and the bars indicate the S.E.M. Where a bar is not seen, the S.E.M. was less than the range of the point.
Table 1. Percentage of viable cells after infection with HSV-2

<table>
<thead>
<tr>
<th>Dose of HSV-2</th>
<th>Total peritoneal cell population</th>
<th>Adherent macrophages</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>10 h</td>
<td>36 h</td>
</tr>
<tr>
<td>Medium</td>
<td>87.3 ± 0.9*</td>
<td>85.1 ± 2.3</td>
</tr>
<tr>
<td>3 × 10^6 p.f.u./ml</td>
<td>85.1 ± 1.2</td>
<td>85.0 ± 2.2</td>
</tr>
<tr>
<td>10^7 p.f.u./ml</td>
<td>87.0 ± 0.7</td>
<td>84.1 ± 3.3</td>
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* Mean ± S.D. of three cultures.

Infection was examined, essentially the same pattern was seen (Fig. 1 c).

To examine whether the decline in respiratory burst capacity seen with high doses of infectious HSV-2 was due to a cytolytic effect of the virus, cultures of total peritoneal cells (3 × 10^6 cells) or adherent MΦ (about 5 × 10^6 MΦ) were infected with HSV-2 and examined for viability by trypan blue exclusion after 10 and 36 h. As seen from Table 1 even the high dose of virus (10^7 p.f.u./ml, equivalent to an m.o.i. of 3 for the total peritoneal cell population and about 20 for adherent MΦ) did not reduce the cell viability considerably. However at the high virus dose the adherent MΦ appeared more swollen than uninfected cells.

Respiratory burst of peritoneal MΦ treated with IFN-α/β and TNF-α

Infection of peritoneal MΦ with 10^4 to 3.2 × 10^7 p.f.u./ml of HSV-2 for 10 h resulted in an increased capability of the cells for a respiratory burst, in this experiment peaking at an infectious dose of 10^7 p.f.u./ml (m.o.i. of 2.7; Fig. 2). Parallel cultures treated with 160 IU/ml of crude macrophage-produced IFN-α/β (IFN-MΦ), 500 IU/ml of IFN-α or 500 U/ml of TNF-α were also tested for respiratory burst (Fig. 2). The IFNs induced a respiratory burst capacity in the peritoneal cells which was comparable to that induced by the virus. The two IFN preparations were not equally efficient in priming the peritoneal cells for a respiratory burst. TNF-α was not able to prime the cells for an augmented chemiluminescence response; in fact the respiratory burst after treatment with higher doses of TNF-α was repeatedly and significantly diminished. Neither the cytokines nor HSV-2 induced any spontaneous respiratory burst in the peritoneal cells during the incubation period (data not shown).

Synergistic interactions in the priming of peritoneal MΦ for a respiratory burst by IFN-α/β and TNF-α

Because both IFN-α/β and TNF-α are produced during infection with HSV-2 and IFN-α/β has been shown previously to be involved in the HSV-2-induced acti-
autocrine HSV-2-induced macrophage activation

Fig. 3. Interaction of IFN-α and TNF-α in the priming of mouse peritoneal cells for an increased respiratory burst. Peritoneal cells were treated with 500 IU/ml of IFN-α (●), 500 U/ml of TNF-α (□), both of the cytokines (■) or medium alone (○) and assayed for PMA-triggered luminol-dependent chemiluminescence response after 10 h of incubation. The kinetics of the PMA-triggered response is depicted. Points represent the mean value from five cultures (controls from seven cultures), and the bars indicate the S.E.M. Where a bar is not seen, the S.E.M. was less than the range of the point.

Fig. 4. Two-dimensional titration of the synergistic interactions between IFN-α and TNF-α in the priming of peritoneal cells for an increased respiratory burst. Mouse peritoneal cells were cultured for 10 h in various combinations of IFN-α and TNF-α. At the end of the incubation period the PMA-triggered luminol-dependent chemiluminescence response was measured and depicted as columns representing the mean response of two cultures, except the control, which represents the mean of five cultures (IFN-α with or without TNF-α), two cultures (TNF-α only) or 13 cultures (controls). Bars indicate the S.E.M. Where a bar is not seen, the S.E.M. was less than the range of the point.

concentrations of IFN-α and/or TNF-α, and the respiratory burst potential of the cells was measured (Fig. 4). From this experiment it is seen that IFN-α induced a concentration-dependent activation of the peritoneal Mϕ, and a clear synergistic interaction between the two cytokines was seen in the whole concentration range examined. The synergistic interaction was most pronounced with the higher concentrations (100 and 1000 U/ml) of TNF-α, and intermediate concentration (100 IU/ml) of IFN-α (for both combinations 2P < 0.05).

The kinetics of the synergistic priming by IFN-α and TNF-α of peritoneal cells for an increased respiratory burst was examined by treating the cells with the cytokines at various time periods before the assay for chemiluminescence (Fig. 5). The kinetics of the IFN-α and the combined IFN-α- and TNF-α-induced enhancement of the respiratory burst were identical, with a significantly increased response and synergism (2P = 0.0001) between the two cytokines observed as early as 4 h and a maximal response seen in cells cultured for 12 h with the cytokines. This indicates that the mechanism of the synergistic activation is similar to that of IFN-α alone. TNF-α reduced the response (2P = 0.0007), and the reduction was at a steady state level from 6 h after addition of TNF-α.

In a few experiments the respiratory burst was slightly increased after treatment with higher concentrations of TNF-α (for instance the experiment depicted in Fig. 4). To elucidate this phenomenon peritoneal cells were cultured with 500 U/ml of TNF-α, 200 NU/ml of a rabbit antiserum to IFN-α/β, or the combination of these for 10 h in a series of six experiments. In one of
Fig. 6. Neutralization by antibodies to IFN-α/β of the occasionally appearing activation of Mφ by TNF-α. Mouse peritoneal cells were treated with 500 U/ml of TNF-α, 100 NU/ml of rabbit anti-IFN-α/β antiserum or a combination of these for 10 h. The cells were then assayed for PMA-triggered luminol-dependent chemiluminescence response. The columns represent the mean response of five cultures, bars indicating the s.e.m.

Neutralization of IFN-α/β and TNF-α in the HSV-2-induced priming of Mφ for a respiratory burst

The possible involvement of both IFN-α/β and TNF-α produced early after HSV-2 infection of peritoneal Mφ in the increased capacity for a respiratory burst was investigated by neutralizing antibodies to IFN-α/β and TNF-α. Peritoneal cells were infected with 3 × 10⁶ p.f.u./ml of HSV-2 (m.o.i. 0.8), incubated with 100 NU/ml of anti-IFN-α/β and/or anti-TNF-α antibodies and assayed for chemiluminescence response 10 h later (Fig. 7a). From the results it is clearly seen that IFN-α/β, and also TNF-α, produced during the infection is involved in the activation of the cells. Furthermore, an effect of combination of the antibodies was seen, showing that synergistic activation of the cells did take place in the HSV-2-infected cultures.

In a similar experiment (Fig. 7b), except that 20-fold higher concentrations of antibodies (2000 NU/ml) were used, it was found that the HSV-2-induced capacity for a respiratory burst was completely abolished by the antiserum to IFN-α/β, indicating that IFN production is
essential for the activation process, which is further augmented by the presence of TNF-α.

**Discussion**

We are currently analysing the reciprocal interplay between Mφ and cytokines in natural resistance against viral infections. In these studies we are taking advantage of a mouse model of generalized HSV-2 infection, in which the intrinsic antiviral activity and early IFN-α/β production by Mφ strongly correlates with resistance to the infection (Mogensen, 1977; Pedersen et al., 1983; Ellermann-Eriksen et al., 1986b). Resistance to HSV-2 infection is influenced by an X-linked gene or gene complex, and this pattern of inheritance is also found in the early IFN-α/β production in response to the virus. This has also been noted for HSV-1 (Zawatsky et al., 1982), emphasizing that early IFN production could be important for restriction of HSV infections. Later we showed that the sensitivity to IFN-α/β of murine fibroblasts and Mφ in the same system is also genetically determined, with cells from resistant mice being more sensitive to IFN than cells from more virus-susceptible mice (Ellermann-Eriksen et al., 1986a, 1989). Recently we have described increased capacity for a respiratory burst by Mφ during infection of these cells with HSV-2 (Mogensen et al., 1989). Mφ from the virus-resistant mouse strain showed a higher response than cells from the susceptible strain and part of this HSV-2-induced activation of Mφ was shown to be dependent on autocrine IFN-α/β production. Previously IFN has been shown to be a priming substance for a respiratory burst by peritoneal Mφ and peripheral blood leukocytes (Jarstrand & Einhorn, 1981; Ito et al., 1985; Müller et al., 1987).

In the present paper we examined whether another monokine, namely TNF-α, is involved in an autocrine manner in activation of Mφ during HSV-2 infection. Both of the cytokines IFN-α/β and TNF-α were produced in significant amounts in cultures of peritoneal cells in response to infectious and u.v.-inactivated HSV-2, but not in response to heat-inactivated virus. This pattern is compatible with previous observations showing that glycoprotein D of HSV-1 is a major inducer of the production of IFN-α (Lebon, 1985). Previously we have shown Mφ to be the only cell subset in the peritoneal cavity with a significant production of IFN-α/β early after HSV-2 infection, and to be largely responsible for the respiratory burst (Ellermann-Eriksen et al., 1986b; Mogensen et al., 1989). In the context of cytokine production and respiratory burst it should be noted that a close correlation was found between the ability of infectious and u.v.-inactivated virus to induce IFN-α/β and TNF-α and to prime for a respiratory burst and the inability of heat-inactivated virus in both respects. The swollen appearance of Mφ infected with high doses of virus might indicate that a cytotoxic effect of the infectious virus could be responsible for the decline in respiratory burst capacity seen with the highest virus dose. Previous studies in the laboratory have shown that only a minor fraction of Mφ support productive replication of HSV-2 (Mogensen, 1979).

In contrast to findings by others (Shparber & Nathan, 1986; Ding & Nathan, 1987; Ferrante et al., 1988; Wewers et al., 1990) the present study shows that exogenously added TNF-α slightly but clearly and significantly decreases the respiratory burst capacity of peritoneal Mφ. An increased respiratory burst after treatment with TNF-α was seen only occasionally, but this effect could be neutralized by an antiserum to IFN-α/β, indicating that TNF-α-induced activation is dependent on spontaneous production of endogenous IFN-α/β during culture. It remains to be determined whether activation of peritoneal Mφ by TNF in general is dependent on IFN. The rather late appearance of the maximal capacity for a respiratory burst (approximately 40 h) seen in the studies by Shparber & Nathan (1986) and Ding & Nathan (1987) as compared with the one seen with IFN-α/β in our system (8 to 12 h) could point to spontaneous production of IFN-γ, perhaps by natural killer cells (Bancroft et al., 1989), or other cytokines playing a role in their system. Similarly IFN-γ has been found to be involved in an interleukin-4-induced activation of Mφ (Bhaskaran et al., 1992), and to be a prerequisite for activation of mouse peritoneal Mφ by interleukin-4 (S. Ellermann-Eriksen, unpublished). In the present study independent activation of Mφ by TNF-α was not seen over a 4 day period.

There is increasing support, both theoretically and experimentally, for the concept of a cytokine network. Cytokines have been shown to exert various functional effects in different situations, to induce or inhibit the induction of each other, and to interfere with or modulate the effects of other cytokines (Balkwill & Burke, 1989; Thomson, 1991). In accordance with these interactions combined treatment with IFN-α and TNF-α showed a synergistic effect, which was especially prominent when the Mφ were subjected to intermediate concentrations of IFN-α and relatively high concentrations of TNF-α. The priming effect induced by TNF-α was dependent on the presence of IFN-α/β; without IFN the Mφ respiratory burst capacity was slightly inhibited by TNF. TNF-α interacts with several other cytokines and monokines. For instance, it induces the production of interleukin-6 (Kohase et al., 1986) and synergizes with IFN-γ (Trinchieri et al., 1986; Arenzana-Seisdedos et al., 1988). Whether TNF-α induces synthesis of IFN-α/β has been discussed. This has been described in some systems in
which TNF-α induces antiviral activity (Ito & O’Malley, 1987; Reis et al., 1989; Jacobsen et al., 1989), but not in others (Wong & Goeddel, 1986; Mestan et al., 1986), indicating that IFN-α/β production in response to TNF-α is not a dominant feature of TNF-α. An antiviral effect of TNF-α varies in different systems. For instance an antiviral effect against HSV-2, vesicular stomatitis virus and encephalomyocarditis virus is observed in several cell lines, including mouse embryonic fibroblasts, but not in others, for instance mouse L929 cells (Wong & Goeddel, 1986; Mestan et al., 1986; S. Ellermann-Eriksen, unpublished). Especially when focusing on antiviral effect, TNF-α and IFN-α/β have shown synergistic interactions in fibroblasts (Mestan et al., 1986; Reis et al., 1989; S. Ellermann-Eriksen, unpublished). As regards growth inhibition, a synergistic activity of the two cytokines has been described in some fibroblast and macrophage/monocyte-like cell lines (Orita et al., 1987; Onozaki et al., 1988). Among all these interactions between the two cytokines, a synergism like the one described in this study, between the slightly negatively acting TNF-α and the inducing IFN-α resulting in an approximately doubled inducing effect, is seldom seen.

Subsequently the role of the two monokines, produced by Mφ upon HSV-2 infection, in activation of the Mφ themselves during the infection was investigated. Using moderate amounts of neutralizing antibodies, which did not neutralize all of the activity produced, it appeared that IFN-α/β and TNF-α were both involved in the process, and that a synergistic interaction between them, as described for exogenous addition, occurred in the HSV-2-infected cultures. With high amounts of antiserum, all of the local activity of the monokines was neutralized, revealing that IFN production is a sine qua non for the HSV-2-induced activation of Mφ. This was not found in our earlier study (Mogensen et al., 1989), probably because an excess of antibody is necessary for neutralizing all of the locally produced cytokine in an autocrine system.

Collectively the data indicate the existence of a reciprocal macrophage-monokine alliance in the natural resistance against HSV-2 infections. Mφ are ubiquitous cells strategically placed to monitor most organs of the body. Furthermore, these cells are strongly recruited into infectious foci. The ability of Mφ to react to HSV-2 infection with the prompt production of IFN-α/β and TNF-α and the ability of these monokines in turn to activate Mφ synergistically in an autocrine manner thus represent a self-strengthening system with great antiviral potential.

I thank Birthe Soby for excellent technical assistance, and Soren C. Mogensen for helpful discussions and review of the manuscript. This work was supported by the Aarhus University Research Foundation, grant no. F-1991-LF 1-57, and by the Danish Medical Research Council, grant no. 12-1622-1.

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(Received 9 February 1993; Accepted 28 May 1993)