Herpes simplex virus (HSV) is a human pathogenic virus that infects through mucocutaneous membranes and is able to replicate in most cell types (Whitley, 2001). After a primary HSV infection, the virus establishes latent infections in neurons, from where it can be reactivated in response to various forms of stress. HSV infections can give rise to gingivostomatitis, cold sores and encephalitis (HSV-1), as well as genital and neonatal herpes (HSV-2).

The host reaction to infection is a highly regulated response aimed at eliminating the infectious agent from the organism. Cytokines are soluble secreted factors that play important roles in regulation of the immune response. With respect to virus infections, the interferons (IFNs), a special group of cytokines, have been ascribed particularly important roles (Muller et al., 1994; Mikloska & Cunningham, 2001; Salazar-Mather et al., 2002). IFN-α/β, or type I IFNs, are produced rapidly after infection (Ellermann-Eriksen, 1993; Malmgaard et al., 2002) and form part of the very first line of defence (Stark et al., 1998). IFN-γ is produced both in the early stages of infection by natural killer cells and at later stages by activated T cells (Boehm et al., 1997). IFN-γ is a strong activator of macrophages (Boehm et al., 1997; Paludan et al., 1998; Malmgaard et al., 2000) and is also involved in driving the development of CD4+ T cells towards a Th1 response (Boehm et al., 1997). Not surprisingly, IFN-γ is crucial for elimination of many virus infections, including HSV infections (Kodukula et al., 1999; Harandi et al., 2001; Mikloska & Cunningham, 2001). In this work, we have investigated how expression of IFN-γ is regulated during the early stages of HSV infection in a peritoneal cell culture system containing a mixture of leukocytes.

In order to examine how IFN-γ production is regulated during HSV-2 infection, we first examined the expression of IFN-α/β, interleukin (IL)-12 and IL-18, all cytokines reported to play a role in the regulation of IFN-γ production (Orange & Biron, 1996; Nakamura et al., 1989; Pien et al., 2000; Nguyen et al., 2002). Thioglycolate-elicited peritoneal cells (PCs) were harvested from the peritoneal cavity of 8-week-old female BALB/c mice and cultured in RPMI 1640 medium supplemented with 5% foetal calf serum (BioWhittaker). The following day, the cells were infected with the MS strain of HSV-2 (3 x 10^6 p.f.u. ml⁻¹) and incubated for different intervals up to 24 h at 37 °C. Supernatants were harvested and cytokine levels measured by ELISA (IL-12, IL-18 and IFN-γ) or bioassay (IFN-α/β). As seen in Fig. 1(a), IFN-α/β production was induced within 4 h of infection. IL-12p40 was also induced by HSV-2 infection with a significant increase observed after 8 h of infection (Fig. 1b). For both cytokines, the levels increased continuously throughout the 24 h of the experiment. IL-18 was produced constitutively in amounts well above the detection limit (Fig. 1c) and HSV-2 infection did not affect the production of IL-18 in PCs. This finding, however, does not exclude enhanced IL-18 signalling following HSV-2 infection, since we have previously reported that expression of both the IL-18 receptor and the pro-IL-18-cleaving enzyme caspase-1 are induced by HSV (Paludan et al., 2002). In order to correlate the induction of IFN-γ with expression of the cytokines shown in Fig. 1(a–c), we also examined the kinetics of expression of IFN-γ. Interestingly, accumulation of IFN-γ was detectable after 12 h of HSV-2 infection (Fig. 1d) and hence occurred with delayed kinetics compared with IFN-α/β and IL-18. These findings therefore...
indicate a possible role for these cytokines in HSV-2-induced IFN-\(\gamma\) expression.

In the next set of experiments, we wanted to examine whether type I IFNs, IL-12 and IL-18 did in fact stimulate production of IFN-\(\gamma\) in the cell population used. Elicited PCs were harvested, cultured in RPMI and treated with recombinant cytokines. As seen in Fig. 2, none of the cytokines alone was a potent inducer of IFN-\(\gamma\) expression, with only IL-12 stimulating detectable IFN-\(\gamma\) production. The ability of IL-12 to promote IFN-\(\gamma\) production was strongly augmented by IL-18 and modestly by IFN-\(\alpha\). Stimulating the cells concomitantly with IFN-\(\alpha\) and IL-18 only marginally affected IFN-\(\gamma\) expression. When the cells received all three stimuli, we observed high levels of IFN-\(\gamma\), although these were lower than in response to a combination of IL-12 and IL-18 alone. These findings thus show that IFN-\(\alpha\), IL-12 and IL-18 do induce expression of IFN-\(\gamma\) in thioglycolate-activated PCs from BALB/c mice, and also confirm previous reports in the literature that IFN-\(\alpha/b\) can both promote and inhibit production of IFN-\(\gamma\), depending on the context in which it acts (Cousens et al., 1997; Nguyen et al., 2002).

In order to investigate whether IFN-\(\alpha/b\), IL-12 and IL-18 play a role in the induction of IFN-\(\gamma\) production during HSV-2 infection, we infected PCs with virus and cotreated

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**Fig. 1.** Expression of cytokines during HSV-2 infection in vitro. Thioglycollate-elicited PCs were harvested from BALB/c mice and left overnight in culture to settle. The cells were infected with HSV-2 (3 \(\times\) 10^6 p.f.u. ml\(^{-1}\); m.o.i. 1-7) (closed circles) or left untreated (open triangles). Supernatants were harvested after the indicated periods of infection for measurement of IFN-\(\alpha/b\) (a), IL-12p40 (b), IL-18 (c) and IFN-\(\gamma\) (d). The results are shown as the mean of measurements from triplicate cultures \(\pm\) SEM. Similar results were seen in three independent experiments.

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**Fig. 2.** Induction of IFN-\(\gamma\) production by recombinant cytokines. PCs harvested from BALB/c mice were treated in vitro for 24 h with recombinant cytokines at the following concentrations: 1 ng IL-12p70 ml\(^{-1}\), 1 ng IL-18 ml\(^{-1}\) and 2 \(\times\) 10^8 U IFN-\(\gamma\) ml\(^{-1}\). Supernatants were harvested and IFN-\(\gamma\) levels were measured by ELISA. The results are shown as the mean of measurements from triplicate cultures \(\pm\) SEM. Similar results were obtained in three independent experiments.
with either neutralizing antibodies against IL-12p40, IL-18 or IFN-α/β, or with control antibodies. Depletion of the culture medium for functional IL-12p40 or IFN-α/β reduced the production of IFN-γ by 85–90% (Fig. 3). Elimination of IL-18 also reduced HSV-2-induced IFN-γ production, although not to the same extent. By using combinations of neutralizing antibodies, we found that the absence of IL-12 and any or both of the two other cytokines totally inhibited virus-induced expression of IFN-γ. When IL-18 and IFN-α/β were neutralized, IFN-γ production was inhibited by between 85 and 95%. Taken together, these results show that IL-12, IL-18 and IFN-α/β all play a role in HSV-induced IFN-γ production and also suggest that these three cytokines define the major IFN-γ-inducing cytokines during the early phase of infection, since no residual IFN-γ production was detected when all three were neutralized. It should be noted that we cannot formally rule out an involvement of IL-23, since the p40 subunit of IL-12 is also part of IL-23, which is known to promote IFN-γ expression (Lankford & Frucht, 2003). However, this cytokine primarily targets memory T cells and hence is unlikely to play any major role in the early IFN-γ response to virus infections.

In this study we have examined how the production of IFN-γ is regulated during the early stages of HSV-2 infection. We found that a complex cytokine network is involved, with all of the cytokines IFN-α/β, IL-12 and IL-18 playing non-redundant roles. IL-12 and IL-18 have long been considered to be the prime inducers of IFN-γ (Okamura et al., 1998). However, more recent work has unveiled a more complex picture, with many other cytokines also being able to stimulate IFN-γ production (Carson et al., 1995; Oppmann et al., 2000; Nguyen et al., 2002; Pflanz et al., 2002). The present findings by us and previous reports by others (Sareneva et al., 1998; Nguyen et al., 2002; Pien et al., 2002) suggest that IFN-α/β is a central regulator of IFN-γ production in the context of virus infections. The finding that IFN-α/β is involved in regulation of IFN-γ production sheds new light on the function of type I IFNs, which appear to be important not only for the initial antiviral response, but also for shaping of the subsequent immune response.

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