Absence of tumour necrosis factor facilitates primary and recurrent herpes simplex virus-1 infections

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Tumour necrosis factor (TNF) is an important cytokine in the innate immune response against various infections, including herpes simplex virus (HSV) infection. It has recently become a molecular target of anti-cytokine treatment in certain inflammatory diseases. TNF depletion resulted in a more rapid emergence of infectious HSV-1 in the explant cultures of latently infected trigeminal ganglia (TG), compared with controls. To further evaluate the importance of TNF in the host’s defence responses against HSV-1, TNF-knockout mice were challenged via scarified cornea. These mice were more susceptible to primary acute corneal HSV-1 infection than controls, as manifested by an increased mortality rate and higher infectious virus titres in the eyes and TG, indicating that TNF is critical for defence during acute HSV infection. These results imply that the administration of anti-inflammatory TNF antagonists might facilitate the propagation of infectious HSV, resulting in an exacerbation of primary and recurrent acute lesions.

Herpes simplex virus (HSV)-1 is a human alphaherpesvirus commonly found throughout the world. Primary HSV-1 infection is usually associated with only mild clinical manifestations, but it can occasionally be fatal. Despite eliciting a vigorous immune response during primary infection, HSV-1 establishes latent infections within peripheral neurons. Various external stimuli (e.g. stress) induce viral gene expression, genome replication and, ultimately, production of infectious progeny virus (Feldman et al., 2002; Jones, 2003). This process, reactivation, is usually recognized by a recurrent disease and/or virus transmission. Recurrent acute lesions are usually milder than the primary one, but often severe in immunologically compromised patients. Asymptomatic shedding of infectious virus provides a source of infection (Whitley, 2001). Despite the huge accumulation of clinical and experimental studies on the host defence responses against HSV (Koelle & Corey, 2003; Wagner & Bloom, 1997), no single host factor, nor set of host factors capable of preventing virus reactivation/recrudescence has been identified.

The reactivation of latent HSV-1 within the trigeminal ganglia (TG) causes recurrent debilitating keratitis. Studies of mouse TG latency models established that the presence of the HSV-1 genome within TG in vivo is associated with mononuclear infiltrates (Halford et al., 1996; Liu et al., 1996; Shimeld et al., 1997) and elevated production of proinflammatory cytokines, e.g. tumour necrosis factor (TNF), interleukin-6 (IL-6) and interferon-γ (IFN-γ) (Cantin et al., 1995; Halford et al., 1996; Liu et al., 1996; Shimeld et al., 1997, 1999). The persistently elevated expression of cytokines during latency is attributed to infrequent spontaneous virus reactivation (Chen et al., 2000; Feldman et al., 2002; Halford et al., 1996). These observations suggest that these proinflammatory cytokines are involved in the host defence response against HSV-1 reactivation. That the addition of syngeneic splenocytes to TG explant cultures significantly delays HSV-1 reactivation from latency (Noisakran & Carr, 1999) further indicates that immunological mediators suppress HSV-1 reactivation.

TNF, while recognized as a critically important antiviral cytokine (Herbein & O’Brien, 2000; Rossol-Voth et al., 1991; Wong & Goeddel, 1986), has been blamed for unnecessary inflammation that can be harmful to the host, and thus has become a molecular target of anti-cytokine therapy. TNF antagonists (anti-TNF mAbs and ‘etanercept’, a dimeric molecule that contains two p75 TNF-receptor extracellular domains attached to the Fc portion of human IgG1) have been introduced to treat patients with rheumatoid arthritis and several other chronic inflammatory diseases (Fox, 2000). These new therapies provide excellent control of inflammation, although they increase the risk of serious infections involving intracellular bacteria (Gardam et al., 2003). Since HSV infection is common in patients of all ages, anti-TNF therapy may increase recurrent HSV diseases. The observation that clinical application of TNF antagonists is in fact associated with infectious complications urged us to study, in more depth, the effects of TNF on HSV infection, especially as a host defence factor. In this study, we analysed the effects of TNF deprivation on HSV-1 reactivation from latency.
in vitro, and examined HSV-1-infected TNF-knockout (TNF-ko) mice in vivo.

The HSV-1 7401H strain, a clinical isolate, was propagated and assayed on Vero cells grown in Eagle’s MEM supplemented with 5% calf serum and kanamycin (60 mg l⁻¹) (Minagawa et al., 1997) before it was used as the challenge virus. For the corneal inoculation (Minagawa et al., 1997), mice were anaesthetized with 50 mg pentobarbital sodium (kg body wt)⁻¹. Both corneas were scarified with a 27-gauge needle, and inoculated with a 7 μl drop of virus suspension. All experimental procedures were approved by the institutional animal care and use committee. Mice were housed in the animal facility at our department under specific pathogen-free conditions.

We first examined the in-vitro HSV-1 reactivation from latently infected TG explant cultures (Fig. 1) after corneal inoculation. After confirming the former report (Noisakran & Carr, 1999) that the addition of mononuclear cells from latently infected mouse spleen delays reactivation (data not shown), we suspected that cytokines, secreted by those cells, would be involved in the defence against HSV reactivation. We examined, therefore, the effects of neutralizing specific proinflammatory cytokines on HSV-1 reactivation from TG explants. In order to monitor TG explant cultures for reactivation, a 50 μl sample of the culture medium was taken from each and transferred to a well in a 24-well plate that contained indicator Vero cells and the appearance of cytopathic effect (CPE) on indicator cells was observed. The supernatant from each well of the TG cultures was sampled daily for the detection of infectious virus. As shown in Fig. 1, depriving the explant of TNF accelerated reactivation; whereas the addition of anti-IL-6 neutralizing antibody to the explant medium delayed it, as well as ultimately reducing the reactivation frequency, as previously reported (Kriesel et al., 1997). Differences between the following groups were statistically significant: anti-TNF-treated and the control, anti-IL-6-treated and the control (P values: 0·01 and 0·03, respectively; Mantel–Cox log-rank test). Depletion of TNF from the TG explant co-cultivated with syngeneic mononuclear spleen cells also accelerated HSV-1 reactivation, while IL-6 depletion delayed and reduced reactivation (data not shown).

To further investigate the role of TNF in HSV-1 infection, 5- or 6-week-old TNF-ko and control mice were inoculated with different doses of infectious virus on the scarified corneas (Fig. 2). Breeding pairs of the mouse strain B6,129-Tnftm1Gk1 (TNF-ko) (Pasparakis et al., 1996) were obtained from the Jackson Laboratory. This strain has a mixed 129Sv x C57BL/6 genetic background. Both 129Sv (H-2b MHC) and C57BL/6 (H-2b) strains show more resistance against HSV infection than the BALB/c (H-2d) strain. We chose the B6 strain as the control because it had been used previously in similar experiments (White et al., 2000).

As shown in Fig. 2, TNF-ko mice were more susceptible to corneal challenge with HSV-1 than were the control animals. The differences between the TNF-ko and B6 groups inoculated with 1 x 10⁶ p.f.u. per eye (P=0·0015; Mantel–Cox log-rank test), and between the TNF-ko and B6 groups inoculated with 5 x 10⁵ p.f.u. per eye (P=0·015; Fisher’s exact test), were significant.

Since TNF plays a central role in innate immunity, the control of virus propagation at the primary entry site would be impaired in TNF-ko mice. Hence, we determined the infectious virus spread and propagation in the eyes, TG, and the brain during the primary phase of corneal infection. A group of three TNF-ko and three control B6 mice that had been inoculated with 5 x 10⁵ p.f.u. of HSV-1 per eye was sacrificed each day on days 1, 3, 5, 8, 11 and 14 after inoculation. Eyes, TG and the brain of each mouse, as well as the liver, spleen, and the adrenal glands, were aseptically removed and assayed for infectious virus titre (Minagawa et al., 1997). As expected, infectious HSV-1 in the inoculated
eyes (Fig. 3A) and in the TG (Fig. 3B) had higher titres and was detected for a more prolonged period in the TNF-ko group than in the control group. The brain from one TNF-ko mouse sacrificed 8 days after inoculation, was found to be positive for HSV-1 (10 p.f.u.). Infectious HSV-1 was not detected in any of the other brains tested. We did not detect any infectious HSV-1 in any of the organs mentioned below: the eyes, TG and the brain from TNF-ko mice or from control animals sacrificed 21 days after inoculation; the spleen, liver and adrenal glands from those TNF-ko mice that were found to be HSV-1-positive in the eyes and/or TG (i.e. those shown in Fig. 3). The course of recovery from primary acute HSV-1 infection in the TNF-ko mice that survived did not differ greatly from that seen among the control mice.

In this paper, we demonstrate that TNF deprivation significantly promoted the reactivation of HSV-1 in latently infected TG explants (Fig. 1). Studies using TNF-ko mice revealed that the absence of TNF increased susceptibility to primary corneal infection (Fig. 2), which coincided with an accelerated and increased infectious virus spread (Fig. 3). Following corneal infection in vivo, the lack of TNF apparently allowed HSV-1 to grow more freely at the site of infection (Fig. 3A) and also at the innervating ganglia (Fig. 3B). Such virus propagation resulted in increased mortality in TNF-ko mice than in controls (Fig. 2).

TNF is required for appropriate immune responses against certain virus antigens (Kasahara et al., 2003; Trevejo et al., 2001). In the case of this HSV-1 study, proper, albeit delayed, adaptive immune responses against the virus were induced, since there was no progressive persistent infection with continuous shedding of infectious virus (Fig. 3) and latency was maintained. Furthermore, in infected TNF-ko mice, both humoral immune responses (i.e. serum neutralizing antibody) and cellular immune responses (i.e. circulating CD4+ and CD8+ T cells that produce IFN-γ in response to HSV antigens) were at least as vigorous as those detected in the control mice (data not shown).

Fig. 2. Survival curves of TNF-ko and control B6 mice after corneal challenge with HSV-1. The groups that were challenged with either 5 x 10^4 (○, TNF-ko) or 1 x 10^6 p.f.u. (■, TNF-ko; □, B6) consisted of five mice, while those that were challenged with 5 x 10^5 p.f.u. (▲, TNF-ko; △, B6) consisted of six mice per group. All mice were 5 or 6 weeks old at the time of corneal challenge. The Mantel–Cox log-rank test and Fisher's exact test were used to determine the significance of the survival curve. P=0.015 for the 5 x 10^5 p.f.u. group and 0.0015 for the 1 x 10^6 p.f.u. group. P<0.05 was considered significant.

Fig. 3. Infectious virus titres from (A) eyes and (B) TG of TNF-ko mice (□) and control B6 mice (●) during the initial acute corneal infection (six organs from three mice per strain per point). All organs of three TNF-ko and three B6 mice sacrificed 21 days after infection were negative for infectious HSV-1. All mice were 5 or 6 weeks old at the time of corneal challenge.
Since deprivation of TNF by neutralizing antibody induced HSV-1 reactivation in the explants (Fig. 1), presence of a small amount of TNF within the ganglion (Shimeld et al., 1997) seems to play an important part in the initial host defence response against recurrent productive infection. As we did not remove infiltrating lymphocytes from the TG explants, the results of our in-vitro study (Fig. 1) did not differentiate between direct and indirect effects of TNF on HSV-infected neurons. The latter indirect effects are likely to be caused by immune effectors under the control of TNF, such as non-cytoidal defensive procedures operated by a small number of T cells in each ganglion. The relative contribution of CD4+ T cells (Minagawa & Yanagi, 2000) and CD8+ T cells (Liu et al., 2000) in the abortion/control of productive recurrent infection (Ghiasi et al., 1999) should be assessed in future studies.

TNF has long been known to exert synergistic antiviral effects together with IFNs (Schmitt et al., 1992; Wong & Goeddel, 1986). In addition, significant contributions of proinflammatory cytokines other than TNF in HSV infections have been reported. IFN-γ secreted by CD8+ T cells in TG has been shown to, at least partially, block HSV-1 reactivation from latency (Liu et al., 2001). Minami et al. reported an increased reactivation rate (indicated by an increased rate of HSV DNA detected from the eye swab samples subjected to PCR) in both IFN-γ−/− and TNF−/− mice (Minami et al., 2002). Anti-IL-6 antibodies were shown to inhibit herpes simplex virus reactivation (Kriessel et al., 1997), but in IL-6-ko mice, both latency and reactivation of HSV-1 were indistinguishable from controls (LeBlanc et al., 1999). Since TNF itself induces IL-6 production, and as both TNF and IL-6 exert neurotropic effects in addition to proinflammatory activities (Hirano, 1998; Zhang & Tracey, 1998), the effects of these cytokines on virus latency/reactivation in the TG may be complex, involving both haematopoietic and neuronal cells within the ganglion. It is of note that one report has demonstrated an acceleration of virus reactivation by TNF (Walev et al., 1995).

In conclusion, the present study indicated that TNF is a critical antiviral cytokine even in the presence of intact adaptive immunity, during both the primary and the reactivating phases of acute productive HSV-1 infection. Patients undergoing anti-TNF therapy should be followed closely for primary and recurrent HSV diseases.

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References


