Dendritic cells presenting equine herpesvirus-1 antigens induce protective anti-viral immunity

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Equine herpesvirus-1 (EHV-1) causes rhino-pneumonitis, abortion and CNS disorders in horses. Using intranasal inoculation, the mouse model of this disease mimics the major pathogenic and clinical features of the equine disease. The aim of this study was to investigate whether murine dendritic cells (DC) can be infected with EHV-1 and whether they can be used as cellular vaccines for the induction of prophylactic anti-EHV-1 immunity. It was found that the DC lines FSDC, D2SC1, 18 (all H-2d) and 80/1 (H-2k), when incubated with the Ab4 strain of EHV-1, do not change their morphology and phenotype but sustain virus replication and induce proliferation of naive, syngeneic T cells. An even stronger proliferation of T cells was seen when DC were used that had been pre-exposed to heat-inactivated virus. DC lines were therefore pulsed with inactivated virus and were then administered intranasally to either BALB/c or C3H mice on days 25, 15 and 5. Control groups received either medium, unpulsed DC or inactivated virus only. Animals were challenged with EHV-1. Whereas mice of control panels showed clinical signs of EHV-1 disease and 27% died, animals immunized with the pulsed DC lines showed only subtle clinical symptoms, lost significantly less weight, exhibited a reduced virus load in their lungs and CNS and did not succumb to the disease during the observation period. These results show that murine DC can present EHV-1 and initiate a protective anti-viral immunity in vivo.

Introduction

Equine herpesvirus-1 (EHV-1) is an important pathogen of horse populations worldwide and is responsible for a variety of clinical problems including respiratory distress, abortion and neurological disorders (for references Crabb & Studdert, 1995). Using intranasal inoculation, a mouse model was established a few years ago, and the pathogenesis of the infection in these mice has features that resemble the natural infection in horses, including a transient viraemia and a pronounced rhino-pneumonitis (Awan et al., 1990; Inazu et al., 1993). After primary infection, alphaherpesviruses (α-Herpesvirinae) are known to remain latent at neuronal sites. Considering the recent detection of EHV-1 in trigeminal ganglia as one site of latency in specific pathogen-free horses and mice (Slater et al., 1994b; Baxi et al., 1996), EHV-1 resembles a classical α-herpesvirus and the mouse model seemed to be a suitable tool to study generalized infections with α-Herpesvirinae in vivo.

In horses and mice, the anti-viral immunity to EHV-1 consists of neutralizing antibodies, natural killing mechanisms and cytotoxic T lymphocytes (CTL). The latter cells are thought to play a key role in protection (Chong et al., 1992; Chong & Duffus 1992; Alber et al., 1995; Allen et al., 1995; Edens et al., 1996), but the exact conditions for most effectively inducing such a protective response are still a matter of debate. When administered in relatively large amounts, live EHV-1, as well as recombinant glycoproteins gB, gC and gD, can confer protection (Tewari et al., 1994, 1995; Osterrieder et al., 1995; Stokes et al., 1996). Adoptive transfer of T lymphocytes has successfully been used to reduce virus replication and provide protection in mice (Azmi & Field, 1993a, b), but it remains unclear which precise role CD4+ and CD8+ T lymphocytes play in the initiation of the protection.

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Dendritic cells (DC) are known to be the most potent initiators of a T cell-dependent immune response (Steinman, 1991), and MHC class I-dependent antigen presentation has generally been shown for DC, both for viral and tumour antigens (Macaetonia et al., 1988; Nair et al., 1992, 1993; Bender et al., 1995; Böhm et al., 1995; Elbe & Stingl, 1995; Majordomo et al., 1995; Bachmann et al., 1996; Hsu et al., 1996; Porgador et al., 1996; Smith et al., 1996; Young & Inaba, 1996). Thus far and to our knowledge, the use of DC to induce anti-viral immunity in vivo has not been reported. The different methods used to obtain DC, mainly from bone marrow, blood and skin, do not always result in fully homogeneous and/or comparable populations. To circumvent this problem, we took advantage of previously established stable, long-term DC lines from mouse skin and spleen (Paglia et al., 1993; Elbe et al., 1994; Girolomoni et al., 1995). With the aim of developing more broadly active and efficient strategies for the induction of prophylactic anti-EHV-1 immunity, we investigated whether DC can stimulate T cell immunity to EHV-1. We report here that EHV-1 infects several murine DC lines productively and that DC pulsed with heat-inactivated virus are able to stimulate naive, syngeneic CD8+ T cells in vitro, and initiate protective immunity against this virus in vivo.

**Methods**

- **Animals.** BALB/c (H-2d) and C3H/HeJ (H-2k) inbred mice (3–4 weeks old) were obtained from the Dept of Animal Breeding, Robert von Oster tag Institute Berlin, Germany and Bomholtgård, Bomholtvej, Denmark, respectively.

- **Cell lines.** The establishment and culture conditions for the DC lines FSDC, D2SC1, DC18 (all H-2d) and 80/1 DC (H-2d) have been described elsewhere (Paglia et al., 1993; Elbe et al., 1994; Girolomoni et al., 1995). The fibrosarcoma cell line L929 (H-2d, CCL1) and the monocyte/macrophage cell line J774A.1 (H-2d, TIB67) were obtained from the ATCC. The mouse keratinocyte cell line PAM212 (H-2d) (Yuspa et al., 1980) was maintained in normal cell culture medium (NCM; RPMI 1640 supplemented with 10% heat-inactivated FCS, 2 mM l-glutamine, 1 mM sodium pyruvate, 0.1 mM non-essential amino acids, 25 mM HEPES, 50 µg/ml gentamicin, 1× antibiotic/antimycotic solution and 50 µM 2-mercaptoethanol, all from Gibco Life Technologies. The rabbit kidney cell line RK-13 (ATCC, CCL37) and the equine dermis cell line ED (ATCC, CCL57), used for propagation of virus, were kindly provided by H. Field, Cambridge, UK and P. Thein, Munich, Germany, respectively.

- **Virus, cell culture and virus assays.** EHV-1 strain Ab4 (Gibson et al., 1992; Telford et al., 1992) was propagated in RK-13 cells, grown in Dulbecco’s modification of Eagle’s MEM (DMEM) with 5% FCS. Thereafter, virus was purified and accumulated by high-speed centrifugation (15000 g, 15 min, Sorvall) and ultra-centrifugation through a 20% sucrose layer (2 h, 100000 g, Beckmann SW 27 rotor), respectively. A virus pellet of 200 ml supernatent was resuspended in 4 ml NCM and filtered sterile (0.45 µm, Sarostar). EHV-1 was titrated by plaque assay using 24-well flat-bottom culture plates seeded with RK-13 cells. Virus was inactivated by heating at 56 °C for 30 min, and then the suspension was tested for surviving virus by plaque assay. The replication of EHV-1 in DC in vitro was detected by infectious centre assays, co-culturing twofold dilutions of DC with permissive RK-13 cells for 36 h before counting the cytopathic lesions. To determine virus replication in the lungs or brains of infected mice, total organs were collected and homogenized in 1 ml tissue culture medium (DMEM and 5% FCS). The suspensions were serially diluted tenfold and virus titres were determined by plaque titration on RK-13 cells. The results were expressed as the geometric mean (p.f.u. per organ) obtained from six mice tested.

**Fig. 1.** Replication of EHV-1 in selected cell lines. (A) 10^4 DC were incubated for 2 h with 10^4 p.f.u. EHV-1 strain AB4 and cultured for 5 days in 1 ml culture medium. The amount of virus that can be rescued from infected DC was monitored daily by co-cultivating an aliquot thereof with RK-13 cells. The amount of virus per DC is presented in (B). All DC lines were infected and propagated the virus over the observation period. Replication is analogous to the mouse fibroblast cell line L929. Compared to RK-13 or ED cells that heavily propagate the virus but are lysed within 48 h (C), replication is low. All data are expressed as mean values ± SD of triplicates per group.
Immunization with dendritic cells

Fig. 2. Flow cytometric analysis of EHV-1-specific proteins and assessment of surface markers on DC upon EHV-1 infection. (A) FSDC were infected with 1 p.f.u. per cell for 2 h and cultured for 5 days as indicated at the top of the peaks. An aliquot of the cells was analysed daily for the surface expression of EHV-1-specific proteins. Faint expression of these glycoproteins can already be detected within 24 h, thereafter the number of EHV-1-positive cells increases quite slowly as indicated besides the x-axis. (B) FSDC were infected with 1–10 p.f.u. per cell, or pulsed with an equivalent amount of inactivated virus for 2 h, cultured for 2 days and analysed for EHV-1-specific proteins. While pulsed cells failed to express EHV-1, the number of EHV-1-positive cells increases with the infectious doses; the slight difference in the fluorescence intensity, however, is not significant. (C) Phenotypic analysis of EHV-1-infected DC. 80/1 DC were infected for 2 h with 100 p.f.u. per cell of live EHV-1 and cultured for 24 h in their culture medium. Thereafter, untreated (control) or EHV-1-infected 80/1 DC were washed and stained with appropriate MAb. Filled profiles illustrate the reactivity of relevant MAb, whereas open profiles represent irrelevant isotype-matched control MAb. Data shown represent one of at least three independent infections.

Infection of DC with EHV-1. To determine whether DC represent a target for EHV-1, 10^6 DC were incubated for 2 h with 10^4 p.f.u. EHV-1 strain AB4. The cells were fixed with formalin at the onset of plaque formation. Serum dilutions and an appropriately diluted FITC-conjugated second-step reagent (goat anti-mouse FITC, Dianova) were incubated for 30 min each. Analysis was performed with an Axiovert S 100 (Zeiss).

Flow cytometry. To determine the expression of surface markers on DC, FITC-conjugated anti-hamster MAb 3E2 (anti-CD54) and 16-10A1 (anti-CD80) were purchased from PharMingen. The anti-mouse MAb 11-4.1 (anti-1-2K*) and 15-5-5S (anti-1-2D*) were obtained from Becton Dickinson and PharMingen, respectively. For the detection of non-labelled MAb anti-1-2K* and anti-1-2D*, the FITC-conjugated

independently. Serum antibody titres were measured by indirect immunofluorescence. Briefly, RK-13 cells in 96-well plates were infected with 50 p.f.u. per well EHV-1 strain AB4. The cells were fixed with formalin at the onset of plaque formation. Serum dilutions and an appropriately diluted FITC-conjugated second-step reagent (goat anti-mouse FITC, Dianova) were incubated for 30 min each. Analysis was performed with an Axiovert S 100 (Zeiss).
second-step reagent goat F(ab’)2 anti-mouse IgG (H+L) (Caltag) was used. In addition, the expression of viral proteins on the surface of infected cells was examined using a polyclonal rabbit anti-EHV-1 serum as described earlier (Slater et al., 1994a). For the determination of surface markers, DC were cultured 8–24 h prior to flow cytometric analysis. For control purposes, cells were incubated with isotype-matched mAbs. Stained cells were analysed using FACScan flow cytometers equipped with LYSYS II and CellQuest software (Becton Dickinson).

**T cell proliferation assay.** 80/1 DC or control cells (L929) were either infected with live EHV-1 (1–100 p.f.u. per cell) or pulsed with an equivalent of heat-inactivated virus for 2 h at 37 °C in 1 ml NCM with occasional agitation. After three washes, cells were X-irradiated (1.5 Gy/min, Philips RT 305) with 40 Gy and seeded into 96-well round-bottom culture plates at 10^5 cells per well. Antigen presenting cell-depleted, naive, syngeneic CD8+ T cells (97–99% purity, as determined by flow cytometry) were obtained according to a protocol described elsewhere (Elbe et al., 1994) and 2 x 10^6 T cells were added to each well in either NCM or NCM supplemented with recombinant mouse IL-7 (6 ng/ml, Genzyme). At days 3, 4 and 5 of culture, cells were pulsed with 3H-TdR (37 kBq per well, Amersham) for 8 h and incorporation of the radionucleotide was assessed by β-scintillation spectroscopy (Packard Instruments). Data are expressed as mean values ± SD of triplicates.

**Vaccination and infection of mice.** On days −25, −15 and −5, DC (FSDC, D2SC1, 18, 80/1) or control cells (PAM212, J774, L929) were pulsed with inactivated virus (5 p.f.u. per cell) for 90 min at 37 °C in NCM with occasional agitation. Subsequently, each mouse was immunized intranasally with 10^6 EHV-1-pulsed cells in a volume of 40 μl medium. Control groups received NCM, the analogous amount of inactivated virus or unpulsed DC only. On day 0, mice were challenged intranasally with 10^5 p.f.u. live EHV-1. Clinical signs as well as the body weight of all animals were monitored daily. To determine the amount of virus replication in lungs or brains, two animals of each group were killed on days 2 and 4, respectively. Experimental groups consisted of six mice each.

**Statistical analysis.** StatView 4.5 was used to evaluate the significance of experimental versus control group data.

**Results**

**Mouse DC can be infected with EHV-1**

Our first set of experiments was designed to investigate whether DC can be infected with EHV-1. As seen in Fig. 1(A), all DC lines propagated EHV-1 throughout the observation period of 5 days. The replication rate per cell was rather low (Fig. 1B). Replication was analogous to the mouse fibroblast cell line L929, but relatively low when compared to permissive rabbit or horse cells (Fig. 1C). Flow cytometric analysis of EHV-1-infected FSDC revealed that some of the cells express viral glycoproteins (Fig. 2A, B). Faint expression of EHV-1-specific proteins was already detected on 8–18% of infected FSDC after 24 h. The amount of proteins expressed as well as the numbers of EHV-1-positive cells (ranging from 15–48%) increased only slowly thereafter (Fig. 2A). Additionally, we observed that the number of EHV-1-positive FSDC was dependent on the amount of virus used for infection; however, minor differences with regard to the amount of protein expressed were not significant. In contrast, FSDC pulsed with heat-inactivated virus failed to express EHV-1 proteins (Fig. 2B).

**EHV-1-pulsed 80/1 DC are potent stimulators of naive, syngeneic CD8+ T cells**

The aim of the following experiments was to investigate the role of DC in stimulating T cell immunity to EHV-1. We have previously shown that the MHC class I/II+/CD80+ DC line 80/1 induces proliferation of naive, allogeneic CD8+ T cells in an MHC class I-restricted fashion in vitro, but does not stimulate proliferation of naive, allogeneic CD4+ T cells (Elbe et al., 1994). The sensitizing power of 80/1 DC in vivo was evidenced by the recent findings that these cells are able to induce transplantation immunity and hapten-specific immune responses (Lenz et al., 1996; Kolesaric et al., 1997). Thus, we felt...
Table 1. Clinical signs observed in mice and control animals after EHV-1 challenge

Groups are listed according to the genetic background of the mouse strains. Minor differences between BALB/c and C3H mice occur in the markedness of the symptoms: while crowding and roughed fur are stronger in BALB/c mice, ataxia was slightly more prominent in C3H mice. Roughed fur is also graded from ‘−’ (no roughness) to ‘+++’ (very strong roughness, affecting the total animal). Data shown summarizes the observations from independent groups of six mice each. Roughed fur and crowding occurred from the first day of infection. Ataxia occurred from day 4 on, so that only two mice in each set of experiments remained (the others had served for determinations of virus titres in lung or brains). Animals died throughout the observation period of 7 days. The number of dead animals includes those euthanized owing to terminal illness. Control animals (BALB/c or C3H) were treated with medium, DC (FSDC or 80/1) or inactivated EHV-1 (ΔEHV-1) alone.

<table>
<thead>
<tr>
<th>Mouse strain/ cell line</th>
<th>Onset of clinical signs (day no.)</th>
<th>Roughed fur</th>
<th>Crowding</th>
<th>Ataxia</th>
<th>No. of dead animals</th>
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<tr>
<td>BALB/c medium</td>
<td>1</td>
<td>++ + (12/12)</td>
<td>12/12</td>
<td>3/0</td>
<td>4/12</td>
</tr>
<tr>
<td>BALB/c FSDC</td>
<td>1</td>
<td>++ + (12/12)</td>
<td>12/12</td>
<td>2/0</td>
<td>5/12</td>
</tr>
<tr>
<td>BALB/c ΔEHV-1</td>
<td>1</td>
<td>++ + (18/18)</td>
<td>18/18</td>
<td>3/0</td>
<td>5/18</td>
</tr>
<tr>
<td>18</td>
<td>2</td>
<td>+ (6/18)</td>
<td>8/18</td>
<td>0/0</td>
<td>1/18</td>
</tr>
<tr>
<td>FSDC</td>
<td>2</td>
<td>+ (7/18)</td>
<td>6/18</td>
<td>0/0</td>
<td>0/18</td>
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<tr>
<td>D25C1</td>
<td>1</td>
<td>++ + (18/18)</td>
<td>18/18</td>
<td>2/0</td>
<td>5/18</td>
</tr>
<tr>
<td>J774</td>
<td>1</td>
<td>++ + (18/18)</td>
<td>18/18</td>
<td>3/0</td>
<td>4/18</td>
</tr>
<tr>
<td>PAM212</td>
<td>1</td>
<td>++ + (18/18)</td>
<td>18/18</td>
<td>2/0</td>
<td>4/18</td>
</tr>
<tr>
<td>C3H medium</td>
<td>1</td>
<td>++ (12/12)</td>
<td>9/12</td>
<td>3/0</td>
<td>4/12</td>
</tr>
<tr>
<td>C3H 80/1DC</td>
<td>1</td>
<td>++ (12/12)</td>
<td>8/12</td>
<td>4/0</td>
<td>3/12</td>
</tr>
<tr>
<td>C3H ΔEHV-1</td>
<td>1</td>
<td>++ (18/18)</td>
<td>12/18</td>
<td>4/0</td>
<td>3/18</td>
</tr>
<tr>
<td>80/1</td>
<td>2</td>
<td>− (0/18)</td>
<td>5/18</td>
<td>0/0</td>
<td>0/18</td>
</tr>
<tr>
<td>L929</td>
<td>1</td>
<td>++ (18/18)</td>
<td>14/18</td>
<td>4/0</td>
<td>4/18</td>
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that this DC line could be an ideal tool to investigate whether EHV-1-treated DC can stimulate naive, syngeneic CD8+ T cells. We further asked whether an actual EHV-1 infection is needed for an efficient presentation of viral antigens by these cells and what amount of virus would provide the best results. For this purpose, 80/1 DC were either infected or pulsed with heat-inactivated virus, testing virus amounts from 1–100 p.f.u. per DC. The infection neither changed their morphology nor influenced the expression of MHC class I and the co-stimulatory molecules CD54 and CD80 (Fig. 2C). Co-culturing with highly purified naive, syngeneic CD8+ T cells revealed that DC pulsed with heat-inactivated virus induced a stronger proliferative T cell response than EHV-1-infected 80/1 DC, with peak responses occurring with 100 p.f.u. per cell at day 4 of co-culture (Fig. 3A, B). To determine whether other MHC class I+II- cells, when infected or pulsed with heat-inactivated EHV-1, are also able to stimulate naive, syngeneic CD8+ T cells in vitro, we tested the MHC class I+ fibrosarcoma L929 in a similar experimental setting as described above. L929 cells infected or pulsed with heat-inactivated EHV-1 failed to induce a significant proliferative response of naive, syngeneic CD8+ T cells (Fig. 3C and data not shown). This shows that MHC class I expression on stimulator cells is not sufficient to induce productive T cell responses and implies that the ability of 80/1 DC to stimulate T cell proliferation in the absence of MHC class II molecules is presumably due to the additional delivery of co-stimulatory signals. Furthermore, the results demonstrate that DC do not require actively replicating virus to load their MHC class I molecules but, instead, inactivated viral proteins brought into DC by non-infectious particles suffice to activate CD8+ T cells. Based on a previous report that IL-7 supports the activation and growth of in vitro antigen-specific CTL precursors (Ferrari et al., 1995), we added recombinant IL-7 to the cultures of 80/1 DC, L929 cells and CD8+ T cells. This led to a more vigorous T cell response to EHV-1-pulsed, but not to unpulsed, 80/1 DC. In contrast, IL-7 failed to augment the T cell response to EHV-1-pulsed L929 cells (Fig. 3C).

Protection against virus challenge following intranasal immunization with EHV-1-pulsed DC

In order to test the in vivo activity of the DC lines, we used two different mouse strains (BALB/c and C3H) corresponding to the genetic background of the DC lines. Since the EHV-1-driven T cell proliferation was superior with inactivated virus, and the use of non-replicating EHV-1 together with DC might offer advantages over live virus preparations (see Discussion), the in vivo experiments were performed with DC pulsed with heat-inactivated EHV-1. We used an immunization protocol
Fig. 4. Mean weights of immunized animals on days 1 to 7 after challenge with infectious EHV-1. Infected mice (A, BALB/c; B, C3H) show two different patterns of reaction. Weight curves of animals immunized with EHV-1-pulsed control cell lines (PAM212, J774, L929) or DC line D2SC1 showed a steady decline in weight. Mice immunized with EHV-1-pulsed DC lines FSDC, 18 and 80/1 showed only moderate loss in mean body weight. Data are expressed as mean values ± SEM of six animals tested independently. Co, heat-inactivated virus alone (control).

Fig. 5. EHV-1 replication in lungs and brains of BALB/c and C3H mice. Data are shown with regard to the maximum replication of the virus on days 2 (A, lungs) or 4 (B, brains). In both organs, infection was significantly reduced by immunization with EHV-1-pulsed DC lines 18, FSDC and 80/1, but not D2SC1. Bars represent geometric mean virus titres per organ with SEM of six mice. Controls were performed with medium alone, FSDC (BALB/c) or 80/1 (C3H) alone or heat-inactivated virus alone. Asterisks indicate *P < 0.05.

Table 2. Appearance of serum antibodies after EHV-1 challenge

Mice were immunized with FSDC pulsed with heat-inactivated virus or treated with medium only as described in Methods. After EHV-1 challenge, immunized mice developed serum antibodies more rapidly and at a significantly higher titre. Data are expressed as mean of four mice for each group.

<table>
<thead>
<tr>
<th>Group</th>
<th>Time (weeks)</th>
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<tbody>
<tr>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Control (medium)</td>
<td>&lt; 1:10</td>
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<tr>
<td>FSDC</td>
<td>&lt; 1:10</td>
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soluble β-Gal and transfected cell lines as a model system for tumour immunity, unless GM-CSF was provided additionally (Paglia et al., 1996). Likewise, none of the control cells, including the macrophage line J774 as an antigen presenting cell effective in secondary responses (Böhm et al., 1995), showed any protective effects (Table 1; Fig. 4). Antibodies in the serum appear only after the observation period of 1 week. The MHC class II* FSDC, however, support the induction of such antibodies (Table 2), demonstrating that humoral immune mechanisms were induced by the antigen-pulsed DC as well.

Discussion

DC are of growing interest for virus-directed immunological research. Considerable evidence exists that DC effectively present viral antigens to T cells, but their permissiveness for EHV-1 was unknown. Using DC lines and the murine model of EHV-1 infection, our results show that murine DC may be sites for virus infections in addition to initiating an antiviral immune reaction.

EHV-1 infected all DC lines tested, but virus replication was low. This correlates with data obtained by flow cytometry, where only part of the cells expressed EHV-1-specific proteins on their surface. Additionally, the infection appears to be nontoxic, since the DC are not lysed and remain viable for >5 days. These data indicate that DC may mediate a kind of low replicating persistent or restricted infection as previously described for herpes simplex virus type 1 in monocytes/macrophages (Linnavouri & Hovi, 1983; Domke-Opitz et al., 1987; Morahan et al., 1989). This is in accordance with the observation that the kinetics of replicating EHV-1 and infectious titres obtained from the infected mouse cell line L929 are much slower than in permissive rabbit or horse cells.

As 80/1 DC express MHC class I but no class II molecules (Elbe et al., 1994), they provided a suitable tool to study the initiation of a CD8+ MHC class I-restricted, EHV-1-specific proliferation of T lymphocytes by DC. Surprisingly, stronger proliferation of CD8+ T cells was obtained in vitro, when the stimulating DC were pulsed with inactivated virus compared to an actual EHV-1 infection of the DC. It remains to be determined whether the inactivated EHV-1 gains access to the classical, cytoplasmic machinery for processing and presentation on MHC class I molecules or whether the DC lines used in this study employ alternative pathways for processing exogenous antigens on MHC class I molecules (reviewed in Jondal et al., 1996; Rock, 1996; Watts, 1997). Indeed, a non-classical MHC class I processing pathway has been previously described for heat-inactivated Sendai virus, which also generated CTL responses in vivo (Liu et al., 1995). It is conceivable that such a mechanism also takes place in our model system. Thus far, the reason(s) for the relatively poor EHV-1 presentation to T cells after infection is(are) not clear because the expression of MHC class I (both H-2Kk and H-2Dk) and co-stimulatory molecules (e.g. CD54, CD80) remained unaffected post-infection. We presume that upon infection with live EHV-1 the amount of MHC-associated ligand is too small to elicit a strong CD8+ T cell-proliferative response from naive T cells.

IL-7 has been described as an activation and growth factor, being able to enhance the induction of CTL in vitro (Ferrari et al., 1995; Welch et al., 1989; Kos & Müllbacher, 1992, 1993) and anti-viral CTL responses in vivo (Leong et al., 1997). Using the MHC class I+/II- DC line 80/1 and naive CD8+ T cells, the T cell stimulation was strongly enhanced when IL-7 was provided to the cultures, confirming the described T cell-activating capacity of IL-7 in the context of antigen presentation by DC. Thus far, IL-7 has not been reported to be synthesized by DC and it remains to be clarified whether it acts on CD8+ cells alone or on DC as well. Since IL-7 induces the secretion of IL-1α, IL-1β, IL-6 and TNF-α in monocytic cells (Alderson et al., 1991) and IL-6 in turn acts as another growth and differentiation factor for CTL (van Snick, 1990), the direct action on DC may not be excluded. The potential involvement of further cytokines in our system remains to be determined. IL-12, for example, is known to stimulate CTL and co-operate with IL-7 in the induction of human CTL (Mehrotra et al., 1993, 1995).

The successful intranasal administration of virus and recombinant vaccines has been reported (Azmi & Field, 1993a; Osterrieder et al., 1995) and seemed a suitable consideration for a DC-based approach with EHV-1, especially since acute infections had been demonstrated in lungs of mice and horses (Awan et al., 1990; Gibson et al., 1992). The productive infection of DC by EHV-1, however, could confere the problem that infected DC, used for subsequent vaccination procedures, would distribute the virus to mice, resulting in an indirect vaccination with undefined amounts of live virus. Our results indicate that it is possible to use DC lines pulsed with a low amount of inactivated virus to elicit a significant immune response in mice and protect them from a lethal challenge with EHV-1. More than this, clinical signs were clearly reduced, as seen from the body weights of the animals. Accordingly, the virus titres in lungs and brains were reduced significantly, the latter reduction arguing for the brain as a secondary target organ. Anti-viral antibodies in the serum were only detectable 2 weeks after the infection and most likely did not contribute to the initial protection mediated by the DC. This is in accordance with earlier reports on intranasal EHV-1 vaccination (Azmi & Field, 1993b; Osterrieder et al., 1995; Bartels et al., 1998), supporting our view that DC primarily elicited a local immune response in this mouse model. Antibodies might, however, contribute to a protection against reinfecions.

A successful vaccination protocol for EHV-1 has been described in mice, using high doses of live or subunit vaccines. While live vaccines lead to a primary infection and might establish latent or persistent infections, the use of virus preparations or recombinant proteins requires amounts of protein too large for a practical approach in horses (Tewari et
Accordingly, we have not chosen the high amount of protein suggested by the in vitro stimulation of naive CD8+ T cells but elected a low amount of heat-inactivated virus in order to avoid any protective effect by the virus preparation itself. Our results show that a strategy using resident DC in vivo might be a much more powerful vaccination tool. In this EHV-1 infection model, conflicting data exist as to whether the immune response is CD4- or CD8-mediated (Azmi & Field 1993a; Tewari et al., 1994). Although the precise mechanism by which the DC-induced protection occurs in vivo is unknown, it is likely that an initial CD8+ T cell activity is responsible for the observed beneficial effects, since at least DC lines 80/1 and 18 are MHC class II+ and therefore not able to activate CD4+ lymphocytes directly. An approach to stimulate one of these T cell subsets with freshly isolated MHC class I+, class II+ DC, while depleting the other by MAbs in vivo might help to elucidate the mechanisms taking part in the induction of this protection.

Although definitive experimental proof is lacking, we propose that intranasally applied DC, aspirated into the tracheo-bronchial system, can migrate via afferent lymphatics to the lymph nodes in numbers sufficient to initiate an antiviral response. Evidence obtained in preliminary migration studies (F. Steinbach, unpublished data) supports this assumption. This concept fits the biology of airway-DC and earlier investigations on the interaction of airway-DC with pathogens (Schon-Hegrad et al., 1991; Holt et al., 1994; McWilliam et al., 1996; Semper & Hartley, 1996). In summary, we have shown that DC pulsed with heat-inactivated EHV-1 virus can successfully be used for in vivo vaccination in mice. The in vitro data using IL-7 raise the possibility that the in vivo responses may even be improved by the addition of IL-7 or yet undefined cytokines supplied as adjuvants or delivered as gene therapy products.

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