Production of interleukin 1 and tumour necrosis factor activities in bronchoalveolar washings following infection of mice by influenza virus

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Mice were infected with influenza A virus by aerosol. Bronchoalveolar washings obtained from infected mice contained interleukin 1 (IL-1) and tumour necrosis factor (TNF) activities. IL-1 was present at day 4 post-infection but not at day 7. TNF activity was present at day 4 and day 7 post-infection. The presence of both these monokines was coincident with increased cell populations in the lungs. In vitro studies demonstrated that macrophages from non-infected mice produce IL-1 and TNF activities in response to live influenza A virus stimulation. These results suggest that a direct interaction between virus and alveolar macrophages leads to IL-1 and TNF production during the course of infection and could account for both the immune responses and the pathology that occur during influenza A virus infection.

Influenza is a major epidemic virus disease in man. Its continuing importance lies in its ability to cause pneumonitis which occurs in previously fit individuals and in the elderly. Infection of mice with mouse-adapted influenza virus strains by aerosol inoculation results in severe disease with alveolar involvement. The pulmonary pathology in mice is similar to that seen in viral influenza pneumonia in man.

The immune response to influenza virus infection has been studied extensively (see review by Ada & Jones, 1986). The specific immune response involves T helper cells, T cytotoxic cells, B cells and macrophages. Alveolar macrophages are also susceptible to infection in vitro although the virus undergoes abortive replication. Macrophages act as antigen-presenting cells and produce cytokines such as interferon which limits the spread of the virus. To our knowledge, no work has been done upon the effect of virus on interleukin 1 (IL-1) and tumour necrosis factor (TNF) production by alveolar macrophages. Both these lymphokines are involved in inflammation and in the regulation of the immune system; TNF also possesses an antiviral activity.

Studies were undertaken to determine whether IL-1 and TNF could be released in vivo into the lungs of mice during the course of influenza virus infection. The virus used in these experiments was the influenza virus strain A/PR/8/34 (H1N1) which had been adapted to Swiss mice by serial respiratory tract infections and then inoculated into the allantoic cavities of 9 day old embryonated chicken eggs (Rudent et al., 1985).

Influenza virus infection of specific pathogen-free female Swiss mice (CD1 strains) was produced by aerosol inoculation of influenza virus (5 × 10^6 EID50) dispersed in fine droplets as described previously (Rudent et al., 1985) in order to obtain a 50% mortality rate after 14 days. Control mice were treated in the same conditions with a virus-free saline aerosol. At 3 h, 6 h, 18 h and on days 4 and 7 post-infection (p.i.) mice were killed, and their lungs inflated by injecting 0.8 ml of RPMI 1640; the bronchoalveolar washings (BW) were rendered cell-free by centrifugation, and individually tested for IL-1 and TNF activities.

For in vitro studies, the BWs from six to 10 mice were pooled. The cells were harvested by centrifugation, adjusted to 10^5/ml in RPMI 1640 containing 5% foetal calf serum (FCS), plated into the wells of a 24 multi-well plate (Falcon) in a volume of 0.5 ml. After a 2 h incubation, non-adherent cells were removed by washing the cells three times with Hanks’ buffered saline solution. The adherent cells, consisting of more than 98% macrophages as shown by esterase staining, were cultured in 0.5 ml of serum-free medium and 0.1 ml of influenza virus suspension was added (10^8 EID50/ml). Control cells were incubated in the presence of 0-1 ml of non-infected allantoic fluid. In some experiments, cells were incubated for 1 h, washed and incubated for an additional 24 h. In other experiments cells were incubated for 24 h with virus. Supernatants were collected and assayed for IL-1 and TNF activities.

IL-1 activity was assayed by measuring [%H]thymidine
Table 1. **TNF production in BWs from influenza virus-infected mice**

<table>
<thead>
<tr>
<th>Time p.i.</th>
<th>TNF activity in cell-free BWs</th>
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<tbody>
<tr>
<td>3 h</td>
<td>0*</td>
</tr>
<tr>
<td>6 h</td>
<td>0</td>
</tr>
<tr>
<td>24 h</td>
<td>0</td>
</tr>
<tr>
<td>4 days</td>
<td>15–63% (3/10)‡</td>
</tr>
<tr>
<td>7 days</td>
<td>59–80% (10/10)§</td>
</tr>
<tr>
<td>Control</td>
<td>0</td>
</tr>
</tbody>
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* Cell-free BWs were tested at a 1:4 dilution.
† Assays were done on specimens from 10 animals.
‡ TNF activity was expressed as percent cytotoxicity on L-929 cells.
§ Numbers in parenthesis indicate the number of mice giving a cytotoxic activity greater than 50%.

IL-1 activity was observed in most BWs (nine of 12) harvested on day 4; on day 7 no IL-1 activity was detected. The response of thymocytes to BW collected at day 7 was lower than that of thymocytes in the presence of medium (Fig. 1). The same observation was made in control BWs. TNF activity appeared on day 4 p.i. in BWs of three mice out of 10. Higher production was obtained in all mice on day 7. No TNF activity was detected in BWs from control mice (Table 1).

Exposure of alveolar macrophages from uninfected mice to influenza virus *in vitro* for 1 h induced the production of IL-1 activity in culture supernatants. Similarly IL-1 activity was produced following a 24 h incubation with the virus. However, only continuous exposure (24 h) of alveolar macrophages to the virus led to TNF production. Neither IL-1 nor TNF activity was found in supernatants from cells cultured with allantoic fluid (Table 2). The data presented here show that alveolar macrophages are able to release IL-1 and TNF under influenza virus stimulation *in vitro* and that both these mediators are released into the lung during the course of infection.

Detection of IL-1 and TNF activities in BWs on day 4 p.i. coincides with an increase in the number of IL-1- and TNF-producing cells and with an increase in virus titre (Rudent *et al.*, 1987). Thus a replication stage would be necessary to trigger IL-1 and TNF release. On day 7 no IL-1 activity was detected, whereas TNF was still present. This suggests either consumption of IL-1 or production of IL-1 inhibitors, as shown by Roberts *et al.* (1986) and as suggested by the reduction of thymocyte proliferation to Con A.

Both IL-1 and TNF in influenza virus infection are involved in specific and non-specific response. The first
consequence of IL-1 and TNF secretion could be the recruitment of cells from the blood compartment, as both mediators exhibit chemotactic properties (Ming et al., 1987; Le & Vilcek, 1987). Among recruited cells the polymorphonuclear leukocytes have been shown to play a protective role by limiting virus spread in the early phase of infection (Fujisawa et al., 1987). IL-1 also stimulates virus-specific T helper cells, which in turn stimulate virus-specific T cytotoxic cells (Ada & Jones, 1986; Mitchell et al., 1985).

TNF antiviral activity has been documented in various models: its secretion into the lung can lead to either destruction of infected cells (Adlerka et al., 1985) or to the induction of antiviral proteins (Mestan et al., 1986; Van Damme et al., 1987). Besides, IL-1 and TNF are pyrogenic (Dinarello et al., 1986) and the fever observed in influenza virus infection is one mechanism involved in virus destruction.

IL-1 and TNF may also be involved in the development of pathological lesions during the course of infection. Recruitment and activation of T cytotoxic cells can lead to the destruction of infected cells and inflammation. Moreover, in a non-specific way, IL-1 or TNF induces the production of proteolytic enzymes and prostaglandin E2 by a number of cells (Bachwich et al., 1987) leading to cell destruction and the desquamation typical of pneumonia, as well as pulmonary vascular damage (Warr & Jakab, 1983).

References


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