The Role of Host Responses in the Recovery of Mice from Sendai Virus Infection

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SUMMARY

The antiviral responses in mice to intranasal inoculation with Sendai virus are described. To investigate the relative importance of the humoral, cell-mediated and interferon responses, the pathogenesis of this infection was studied in animals which were immunocompetent, T cell-deprived or immunosuppressed with cyclophosphamide. Treatment with cyclophosphamide converted the mild, self-limiting infection observed in immunocompetent mice into a severe and frequently lethal pneumonic disease. This was associated with an enhanced interferon response but no detectable antibody or cell-mediated immune response. T cell-deprived mice suffer an infection of intermediate severity associated with an increased interferon response, a normal humoral immune response and no cell-mediated immune response. The implications of these results in relation to the role of the antiviral responses in recovery from Sendai virus infection are discussed.

INTRODUCTION

Previous work (Robinson et al. 1968) with outbred Swiss albino mice demonstrated that Sendai virus normally causes a mild upper respiratory tract infection from which the mice rapidly recover. However, pre-treatment of the mice with cyclophosphamide results in Sendai virus infection being more severe and frequently fatal (Robinson et al. 1969). This increase in severity was found to be associated with increased interferon production but lack of a humoral immune response. Cell-mediated immune (CMI) responses were not investigated at that time. More recently a 51Cr release assay for the measurement of spleen-cell cytotoxicity in Sendai virus-infected mice has been described (Anderson et al. 1977). Application of this technique has shown that a specific, cell-mediated immune response develops within 4 to 5 days after inoculation of Sendai virus, reaches a peak 2 to 3 days later and declines to become undetectable about 3 weeks after inoculation. This specific CMI response has been shown to be T cell-dependent (Anderson et al. 1979).

Inbred CBA mice were used for investigation of the cell-mediated response since histocompatibility had to be achieved between effector spleen cells and target L cells. Therefore, in order to investigate the importance of the CMI response in the recovery of mice from Sendai virus infection we have studied the course of this infection in normal, in cyclophosphamide-treated and in thymectomized CBA mice.
METHODS

Mice. Age- and sex-matched CBA mice of 20 to 25 g, bred at the London Hospital Medical College, were used throughout this study. T cell-deprived (TXBM) mice were prepared as previously described (Anderson, 1978). Briefly, the thymus was removed at four weeks of age. Ten days later the animals were lethally irradiated and immediately reconstituted with syngeneic bone marrow cells. At least 30 days were allowed to elapse before the inoculation of these animals. Mice found to have thymic remnants post mortem were discarded from the study. Indirect immunofluorescent staining for y antigen performed on splenic lymphocytes failed to reveal any y-bearing T cells. Cyclophosphamide-treated mice were injected subcutaneously with 200 mg/kg of the drug on the day before Sendai virus inoculation.

Virus infection and assay. The Sendal strain of parainfluenza 1 virus was propagated in the allantoic cavity of fertile hens' eggs and the same batch of stock virus was used throughout the study. As previously described (Anderson et al. 1977) infection of mice was achieved by intranasal inoculation of 0.1 ml Sendai virus suspension containing 10^4 MKTCDso (monkey kidney tissue culture infective dose) of virus. Prior to inoculation with Sendai virus, sera were obtained from at least four mice from each group and tested to verify the absence of Sendai haemagglutination-inhibiting HI antibody. Suspensions of lung were prepared from four mice per group killed by cervical dislocation at various intervals after inoculation and assayed for virus content in monkey kidney tissue cultures as described by Robinson et al. (1968). Before making the suspensions, the lungs were scored for macroscopic consolidation by the method of Horsfall (1939).

Interferon assay. Lung suspensions were prepared and assayed for interferon as described by Robinson et al. (1968). Briefly, the supernatants of lung suspensions were dialysed at pH 2.2 to inactivate virus and then returned to normal pH. The capacity of these preparations to protect L929 cells from the c.p.e. of vesicular stomatitis virus was then measured.

Histology. Lungs, taken from mice 10 days after inoculation, were inflated with, and then fixed in, cold alcohol. Parallel sections of lung were stained with haematoxylin and eosin and by a modified Una Pappenheim method.

Sendai HI antibody test. Serum was collected by brachial artery puncture of four ether-anesthetized mice per group at various intervals after inoculation with Sendai virus. Sera from TXBM mice were stored individually while those obtained from control intact animals were pooled. The antibody titres of these sera were determined by the haemagglutination inhibition technique. The test was performed in U-bottomed microplates, utilizing 4 haemagglutinating units of Sendai virus and a 0.4% suspension of indicator chick erythrocytes.

Cell-mediated immune response assay. The 51chromium release assay of cytotoxicity described previously by Anderson et al. (1977) was used to detect cell-mediated immunity. L 929 cells were infected with Sendai virus at a multiplicity of 10 MKTCDso/cell and labelled with sodium 51chromate (Radiochemical Centre, Amersham, Bucks.) at a concentration of 10 μCi/10^7 cells. These target cells were incubated in tissue culture microplates with spleen cells obtained from mice at various intervals after inoculation with Sendai virus for 18 h at 37 °C at an effector cell:target cell ratio of 10:1. Spleen cell cytotoxicity was calculated as

\[
\frac{(\text{Lymphocyte induced } 51\text{Cr release} - \text{spontaneous } 51\text{Cr release})}{\text{Total releasable } 51\text{Cr}} \times 100.
\]
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Table 1. The effect of immunosuppression on spleen cell cytotoxicity and serum antibody titres in Sendai virus-infected mice

<table>
<thead>
<tr>
<th>Experimental group</th>
<th>Spleen cell cytotoxicity at 7 days</th>
<th>Serum HI antibody titre at 14 days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Uninfected</td>
<td>22.0 ± 1.7</td>
<td>&lt; 4</td>
</tr>
<tr>
<td>Control + Sendai virus</td>
<td>50.0 ± 1.6</td>
<td>32</td>
</tr>
<tr>
<td>Cyclophosphamide + Sendai virus</td>
<td>21.7 ± 2.9</td>
<td>&lt; 4</td>
</tr>
<tr>
<td>TXBM + Sendai virus</td>
<td>20.9 ± 1.7</td>
<td>32</td>
</tr>
</tbody>
</table>

RESULTS

Evidence of immunosuppression

Table 1 shows the cell-mediated and humoral immune responses to Sendai virus infection in control and immunosuppressed mice. Seven days after infection (p.i.) the spleen cells of control mice had developed appreciable cytotoxicity. The spleen cells of mice treated with cyclophosphamide prior to inoculation were less cytotoxic, showing levels of activity comparable to those in uninfected mice. Similarly TXBM mice inoculated with Sendai virus failed to develop any increase in spleen cell cytotoxicity.

Five days after inoculation of control mice serum antibody could not be detected. In the following 5 days the Sendai HI antibody titre rose to a titre of 32 on day 10 and this level of antibody was maintained for the remaining 4 days of observation. Sendai HI antibody failed to appear in the cyclophosphamide-treated mice during the 14 days p.i. However, TXBM mice developed Sendai HI antibody to a titre of 32 and the time course of the development of this antibody was identical to that in control mice.

Pathology of Sendai virus infection in immunosuppressed mice

No deaths occurred in either the control mice or the TXBM mice during the 14 days after virus infection. However, 60% of the mice which received cyclophosphamide on the day preceding virus inoculation died between 7 and 11 days p.i. No macroscopic lesions appeared in the lungs of Sendai virus-infected control mice. The lungs of TXBM mice began to show consolidation 4 days p.i. The extent of the involvement increased during the following 2 days so that 25 to 50% of each lung was affected. The gross signs persisted for the 2 week observation period and the mean lung score was 1.75 at 14 days p.i. The lungs of TXBM mice examined 5 months later showed no macroscopic lesions. The lungs of the cyclophosphamide-treated mice which died were totally consolidated and the mean lung score of these and the partially involved lungs of the survivors at 14 days was 3.5.

Histological examination of sections of lung taken from control mice 10 days p.i. revealed considerable damage to the bronchiolar epithelium together with slight thickening of the alveolar cell walls. Associated with the damaged bronchi and neighbouring blood vessels there were intense mononuclear cell infiltrates. Sections of lung from mice treated with cyclophosphamide showed extensive consolidation with marked thickening of the alveolar cell walls and heavy infiltration with polymorphonuclear leucocytes, typical of an inflammatory response. The epithelial cells of many bronchi were degenerate but there was none of the mononuclear cell infiltrate seen in control animals. Sections of lung from infected TXBM mice showed widespread damage to the bronchial epithelium; the alveolar cells were thicker than normal and in areas of consolidation were markedly thickened. In addition intense mononuclear cell infiltrates were observed associated with the damaged bronchi and neighbouring blood vessels. Many of the cells of these infiltrates were highly pyroninophilic suggesting that they were B cell-derived.
Fig. 1. Infectious virus recovered from the lungs of normal (●—●) and TXBM (○---○) mice at various intervals after inoculation with Sendai virus. Titres were calculated by the method of Reed & Muench (1938).

Fig. 2. Interferon content of the lungs of normal (●—●) and TXBM (○---○) mice at various intervals after inoculation with Sendai virus. Titres were calculated by the method of Reed & Muench (1938).

Lung virus and interferon

Fig. 1 and 2 show the results of assays of lung virus and interferon content in control and TXBM mice. Four days after inoculation the lungs of control and TXBM mice contained infectious Sendai virus in high titre. The virus persisted at this level for two days in control mice before being rapidly eradicated, so that 8 days p.i. virus could not be detected in the
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lungs of these mice. In TXBM mice the lungs contained high titre virus up to and including
day 8 p.i. although virus was undetectable 11 days p.i. (Fig. 1).

Four days p.i. the lungs of control mice contained interferon at a titre of 4 to 8 (Fig. 2)
This titre persisted during the following 2 days before slowly declining until lungs removed
from control mice 11 days p.i. did not contain detectable interferon. Interferon appeared in
the lungs of TXBM mice at the same time as it did in control mice (Fig. 2). However, the
peak titre occurred at day 6 and thereafter declined at a slower rate than in the control
mice. Significant titres could be detected 11 and 14 days after inoculation but interferon
was not present 21 days p.i.

DISCUSSION

These results indicate that the course of Sendai virus infection in normal CBA mice is
similar to that described previously for random-bred Swiss albino mice (Robinson et al.
1968). One notable difference was the antibody titres in response to infection. The previous
studies showed that serum HI antibody rose to a peak titre of 256 but in CBA mice the
maximum titre observed was 32. This difference occurred despite the antigenic stimulus, as
measured by the infectious virus content of the lungs, being similar in the two types of
mouse. However, the microscopic lung pathology was less severe in CBA mice than that
observed in random-bred mice.

Administration of the drug cyclophosphamide on the day before inoculation of CBA
mice with Sendai virus resulted in a much more severe illness characterized by extensive
lung consolidation in all mice and death in 60%. This was associated with marked immuno-
suppression. No detectable HI antibody was present; there was failure of development of
virus-specific cell-mediated immunity and the absence of mononuclear cell infiltration
indicated a lack of a local immune response in the lungs. Robinson et al. (1969) demonstra-
ted similar results in cyclophosphamide treated, random-bred Swiss albino mice. These
workers showed that the increased severity of infection in animals immunosuppressed with
cyclophosphamide was associated with persistence of high titre Sendai virus in the lung
tissue. This occurred in spite of the continued presence of elevated levels of interferon in the
infected lungs, indicating that interferon alone cannot terminate the virus infection. Rather
it would appear that continued production of interferon is a consequence of continuing
Sendai virus infection.

Mice which had been selectively depleted of T cells by thymectomy and irradiation suf-
f ered an infection of intermediate severity when inoculated with Sendai virus. Although
there were no deaths in this group there was macroscopic and histological evidence of lung
consolidation not found in immunologically intact control animals. This increased pathology
was associated with more prolonged virus replication in the lung. There is unequivocal
evidence that the Sendai virus-specific T cell-mediated immune response did not occur
at all in TXBM mice. However, this TXBM regime did not impair the production of inter-
feron and the presence of interferon in the lung tissue closely paralleled Sendai virus repli-
cation. The antiviral antibody response of T cell-deprived mice appeared to be unimpaired.
This finding was unexpected since other workers have found that the antibody response to
influenza virus infection is largely T cell-dependent (Virelizier et al. 1974; Iwasaki & Nozima,
1977). However, the TXBM regime adopted in the current work was shown to result in the
efficient depletion of T cells; no T antigen-bearing cells were detected on any occasion in the
spleens of TXBM mice. Furthermore, TXBM mice are found to be unable to mount a serum
antibody response to the T cell-dependent antigen, sheep erythrocytes. Previous work
(Blandford et al. 1971; Blandford & Heath, 1974) has shown that normal mice produce
Sendai antibody locally in the lungs and that the synthesis of this local antibody is delayed
and diminished by cyclophosphamide treatment (Blandford, 1975). In the TXBM mice
described here it is likely that local antibody is produced at the site of infection. A considerable mononuclear cell infiltrate appears in the submucosa of these TXBM mice and in the absence of T cells it is likely that many of these locally accumulating lymphoid cells are B cell-derived and may be producing antibody. This possibility is supported by the finding that many of these cells are strongly pyroninophilic.

It would appear that the development of antiviral antibody enabled TXBM mice to recover from an infection shown to be lethal in the absence of immune responses. This strongly suggests that antibody may play a central role in the termination of replication and elimination of Sendai virus from the mouse lung. However TXBM mice suffer an infection of intermediate severity in spite of an apparently normal antibody response to the virus. This implies a role for cell-mediated immune responses in the termination of Sendai virus infection. Indeed, recent work (Yap & Ada, 1978) has shown that cytotoxic T cells accumulate in the lungs of mice infected with influenza virus, while Jennings et al. (1978) have demonstrated a significant contribution of cell-mediated immunity in protection from re-infection with influenza virus. Unfortunately, in the present study, the HI antibody response to Sendai virus in CBA mice was found to be of very low titre, rendering further detailed analysis difficult. In particular it would be interesting to know to which class of immunoglobulin the Sendai HI antibody of TXBM mice belongs. The thymus-independent antibody synthesized by congenitally athymic nude mice infected with Sindbis virus was found to be of the IgM class. Burns & Allison (1975) and Iwasaki & Nozima (1977) have found that only IgM antibodies were synthesized by T cell-depleted mice infected with influenza virus. Our attempts to determine the sensitivity to 2-mercaptoethanol of the HI antibody produced by Sendai virus infection in CBA mice gave equivocal results. Moreover, the titres are too low to permit analysis by either sucrose density gradient centrifugation or gel filtration. Determination of the class of antibody induced in these T cell-depleted mice will require the application of more sensitive techniques such as radioimmunoassay.

Thus, the outcome of Sendai virus infection appears to depend upon a combination of host responses. In the early stages of infection replication of virus may be restricted by the non-specific actions of both interferon and the NK cells previously described in CBA mice (Anderson, 1978). In addition to this holding operation, humoral and cell-mediated immune responses develop and further inhibit virus replication. Finally the immune responses in conjunction with macrophages result in the elimination of virus from the respiratory tract.

REFERENCES


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