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Cell-Mediated Immunity to Sendai Virus Infection in Mice

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The development of a cell-mediated immune response to Sendai virus infection in mice was examined by the use of a ^{51}Cr release assay of cytotoxicity. A low level of "background cytotoxicity" to Sendai virus-infected L cells was found in the spleens of uninfected CBA mice. Spleen cells from Sendai-infected mice showed an elevated level of cytotoxicity against these target cells for a period of 5 weeks, commencing 4 days after infection of the mice. A more transient response was observed in the spleens of mice infected with a serologically distinct virus, the Kunz strain of influenza. This cross-reacting, cell-mediated immune response was intermediate between that observed in unsensitized and Sendai-sensitized spleen cells. The relevance of these cell-mediated immune responses to respiratory tract virus infections is discussed.

Infection of mice with Sendai virus and an avirulent variant of the Kunz strain of influenza virus has proved valuable in the study of the pathogenesis of respiratory viral infection (21, 22). The striking aspect of these infections is the ultimate abrupt termination of viral growth, eradication of the virus, and rapid repair of the damaged mucous membrane. Previous studies have strongly suggested that the specific immune response is predominantly responsible for the termination of these infections (15, 22, 23). The effect mediated by B lymphocyte-derived cells has been shown to be an important component of this immune response, and cells that produce immunoglobulin G antibody may be as important as those that produce immunoglobulin A (3, 4).

It has become clear that in many virus infections cell-mediated immunity may also be an important parameter of the host response to infection. Considerable data have been accumulated about cell-mediated immune responses to a variety of viral infections (1, 2, 12), but little of this has been concerned with infections of the respiratory tract.

Although macrophage migration inhibition, a correlate of cell-mediated immunity, has been observed by many authors after subcutaneous or intraperitoneal inoculation of respiratory viruses (8, 26, 28), intranasal inoculation of such viruses has produced conflicting results (7, 28). More recently, direct assays of lymphocyte-mediated cytotoxicity have demonstrated a cell-mediated immune response to virus infections of the respiratory tract in both experimental animals (5, 27) and man (14, 24).

This paper describes the use of a ^{51}C release assay of cytotoxicity to examine the development of cell-mediated immunity to Sendai virus infection in mice as a preliminary to investigating the role of this response in acute respiratory viral infection.

MATERIALS AND METHODS

Viruses. The Sendai strain of parainfluenza 1 virus and the Kunz strain of influenza A virus were used. Both viruses were propagated in embryonated hens' eggs, and infectivity (50% tissue culture infective doses [$TCID_{50}$] per milliliter) was assayed in monkey kidney tissue cultures. Antibody levels against these viruses were determined by hemagglutination inhibition carried out in microplates.

Infection of mice. CBA mice of 20 to 25 g were used throughout. Prior to each inoculation, sera were obtained from at least four mice from each group and tested to verify the absence of antibodies to the infecting virus.

Mice lightly anesthetized with ether were inoculated intranasally with 0.1 ml of virus suspension containing either $10^{4.0}$ $TCID_{50}$ of Sendai virus or $10^{2.5}$ $TCID_{50}$ of Kunz virus in broth saline. At various intervals after infection, four mice were killed by cervical dislocation and their spleens were removed.

Infection of the mice was confirmed by demonstrating seroconversion 14 days after inoculation.

Spleen cells. Suspensions of spleen cells were prepared in medium 199 with 5% fetal calf serum by the following method.

The spleens from four mice were pooled, chopped into fragments, and pressed through a stainless-steel meshed sieve with a syringe plunger into approximately 5 ml of medium. The resultant cell suspension was held for 2 min to allow large cell clumps to settle, and the supernatant was removed and centrifuged at 2,000 rpm for 5 min.

The contaminating erythrocytes were lysed by incubation with an equal volume of

tris(hydroxymethyl)aminomethane-buffered 0.083% ammonium chloride, pH 7.2, for 5 min at 37°C. The lymphocyte suspension was then centrifuged, washed twice, and resuspended in medium. A sample was removed for cell viability and concentration determination. Suspensions were 75 to 85% viable on the basis of eosin exclusion.

Target cells. L cells, the C3H mouse-derived fibroblast cell line, were used throughout as target cells. The cells were grown to confluence in medium 199 with 10% heat-inactivated fetal calf serum and then maintained in maintenance medium consisting of medium 199 with 5% heat-inactivated fetal calf serum.

For use as infected target cells, confluent monolayers were inoculated with Sendai virus in the ratio of 10 $TCID_{50}$/cell. Hemadsorption performed 24 h after inoculation with this dose of virus showed that virtually every cell was infected.

Chromium labeling. Sendai-infected or uninfected L cells were stripped from the glass with a methylenediaminetetraacetate (Versene)-trypsin mixture, washed once, and resuspended in 1 ml of maintenance medium. Sodium ^{51}Cr (specific activity, 50 to 400 mCi/mg of chromium; 1 mCi/ml in sterile isotonic saline; The Radiochemical Centre Ltd., Amersham, Bucks., England) was added at a concentration of 100 μCi/10^7 cells, and the suspension was incubated for 30 min at 37°C in a shaking water bath. The reaction was stopped by the addition of 1 mg of sodium ascorbate per 10^7 target cells (25). The cells were then washed twice and resuspended in maintenance medium, and a sample was removed for viability and concentration determination. The cells were 98 to 100% viable on the basis of eosin exclusion.

Assay of ^{51}Cr release. During the labeling period, L cells incorporated between 2×10^3 and 4×10^3 cpm of ^{51}Cr per 10^5 cells. Lysis of the cells with distilled water results in the release of 90% of the incorporated ^{51}Cr into the supernatant. The remaining 10% was found to be associated with cell debris adherent to the culture vessel. The amount of ^{51}Cr released by distilled water lysis into the supernatant was regarded as the "total releasable ^{51}Cr" and all other release figures were expressed as a percentage of this total.

Labeled L cells were suspended to a concentration of 10^6 cells/ml in maintenance medium, and samples of 0.1 ml of this suspension were seeded into the wells of tissue culture microplates, which were incubated at 37°C for 1 h to allow a monolayer to form. Nonadherent cells and medium were removed from the wells by rapid inversion.

A volume of 0.2 ml of either maintenance medium or spleen cell suspension was then added to each of the wells, and the plate was sealed and incubated at 37°C. A ratio of 10 spleen cells per target cell was used. The amount of ^{51}Cr released during incubation was determined by removing 0.1 ml of fluid from each well, taking care not to disturb the cell sheet, and the radioactivity present was measured in a Packard Autogamma deep-well scintillation counter. Total releasable ^{51}Cr was determined in parallel by incubating target cells with 0.2 ml of distilled water and removing the standard sample of 0.1 ml from each well for radioactivity counting.

Results were expressed as the mean of at least 10 replicate wells, plus or minus the 95% confidence limits. Student's t was calculated to estimate the significance of results.

RESULTS

Preliminary experiments had shown that both Sendai and Kunz viruses produce infections in CBA mice that are essentially similar to those previously described in outbred Swiss albino and CD1 mice (21, 22). The infected CBA mice showed similar histological changes, and their peak lung virus titers and the first appearance of serum antibody were found to occur at the same time as in the random-bred mice. The cytotoxic activity of spleen cells removed from CBA mice infected with Sendai virus was used to investigate the development of cell-mediated immunity.

Initial experiments demonstrated that the amount of ^{51}Cr released from the target cells depends upon whether the cells are infected and whether they are exposed to normal or to sensitized spleen cells (Fig. 1). Progressive spontaneous release of ^{51}Cr was observed in both Sendai virus-infected and uninfected cells when incubated in maintenance medium alone. After 20 h of incubation, the uninfected cells had released 25% of their radiolabel and the infected cells had released 45%.

Uninfected target cells did not release significantly more ^{51}Cr when incubated with normal spleen cells (Fig. 1B), but infected target cells released 9% more of their total ^{51}Cr when incubated for 20 h with such normal spleen cells (Fig. 1A). This unexpected cytotoxic effect of normal spleen cells on infected target cells was found to be dependent upon the spleen cells being metabolically active; cytotoxicity was reduced to zero when the spleen cells were heat killed prior to incubation.

The most marked cytotoxic effect was observed when sensitized spleen cells from Sendai-infected mice were incubated with Sendai-infected target cells. From Fig. 1A it can be seen that spleen cells obtained from mice infected 6 days previously induced the release of 80% of the ^{51}Cr compared with only 52% when normal spleen cells were incubated with these target cells for a 20-h period. Sensitized spleen cells did not induce a significantly greater release of ^{51}Cr from uninfected target cells. (Fig. 1B).

A visual demonstration of the marked cytotoxic effect of Sendai-sensitized spleen cells is shown in Fig. 2A; this plate also illustrates the lack of reactivity of these same spleen cells with

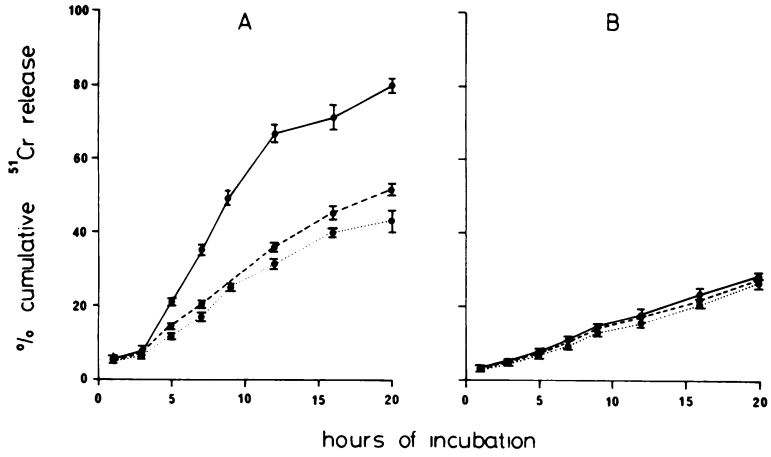

FIG. 1. *Cumulative release of ^{51}Cr from Sendai-infected (A) and uninfected (B) target cells, incubated with: (solid line) Sendai-sensitized spleen cells, (dashed line) unsensitized spleen cells, (dotted line) maintenance medium alone. Mean of 10 replicates ± 95% confidence limits.*

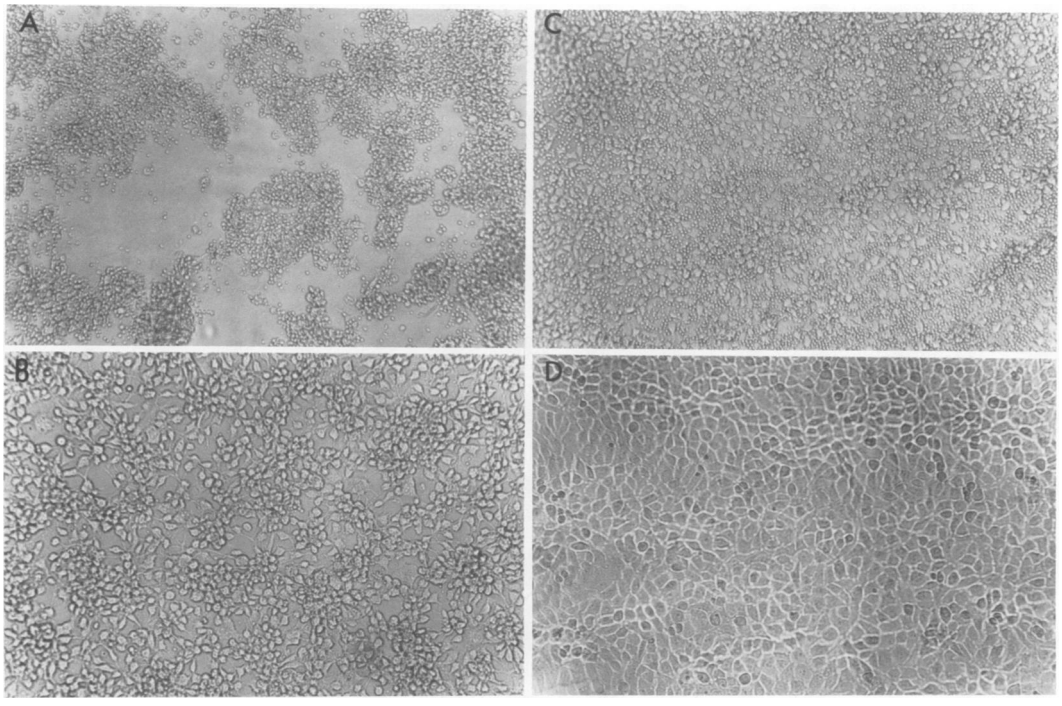

FIG. 2. *Appearance of target cell sheet after 20 h of incubation (×120). (A) Sendai-infected L cells incubated with Sendai-sensitized spleen cells. Note marked cytotoxic effect. (B) Sendai-infected L cells incubated with medium alone and showing viral cytopathic effect. (C) Uninfected L cells incubated with Sendai-sensitized cells. There is no obvious cytotoxic effect. (D) Uninfected L cells incubated with medium alone and showing no abnormality.*

uninfected target cells (Fig. 2C). Sendai virus infection of L cells results in a cytopathic effect (Fig. 2B), and this damage is probably responsible for the elevated spontaneous release of ^{51}Cr from these cells.

Cytotoxic activity of spleen cells during the course of Sendai virus infection of mice. At various intervals after inoculation with Sendai virus, mice were sacrificed and their spleen cells were incubated with Sendai-infected tar-

get cells. In each assay the infected cells were also incubated with spleen cells from normal uninfected mice to provide a measure of background cytotoxicity.

The cytotoxocity found in these experiments was expressed as the "cytotoxic index." This was calculated by subtracting the percentage of ^{51}Cr released spontaneously from infected target cells in the absence of spleen cells from the percentage released from the same target cells incubated with either normal or sensitized spleen cells.

Figure 3 shows that on day 3 after infection spleen cells obtained from Sendai-infected mice were slightly less cytotoxic than normal spleen cells. However, this was followed by a marked increase in their cytotoxic activity, so that the cytotoxic index reached a peak on day 7 after infection. This increased cytotoxicity of the spleen cells gradually declined and had become indistinguishable from background cytotoxicity by day 51 after infection.

Cytotoxic effect of spleen cells obtained from mice infected with an unrelated influenza virus against Sendai virus-infected target cells. Concurrent with the previous experiments, spleen cells were obtained from mice at various intervals after they had been infected with the Kunz strain of influenza A virus. These spleen cells were incubated with Sendai virus-infected target cells, and their cytotoxic effects were determined as described above. The results of these experiments are also shown in Fig. 3. It can be seen that the spleen cells from these mice were also cytotoxic to Sendai virus-infected L cells, but this cytotoxic effect, although appearing at the same time, was less marked and of shorter duration than that observed with the spleen cells from Sendai-infected mice.

Spleen cells from Kunz-infected mice also exhibited a lack of reactivity with uninfected L cells similar to that observed with the spleen cells from Sendai-infected mice.

DISCUSSION

The results of this study show that a detectable cell-mediated immune response occurs after Sendai virus infection in mice. The ^{51}Cr release assay of cell-mediated cytotoxicity provided a sensitive and reliable means of quantifying this response.

Cytotoxic activity was first significantly elevated above the background cytotoxicity present in unsensitized spleen cells 4 days after infection of the mice with Sendai virus and reached a peak 2 days later. These findings are essentially similar to those observed in other experimental systems (5, 9, 17, 29). However, the cytotoxic activity persisted over a longer period in the system described here, remaining significantly above background for 5 weeks after infection. This may reflect an enhanced sensitivity of the ^{51}Cr release assay in this system.

The development of the systemic spleen cell

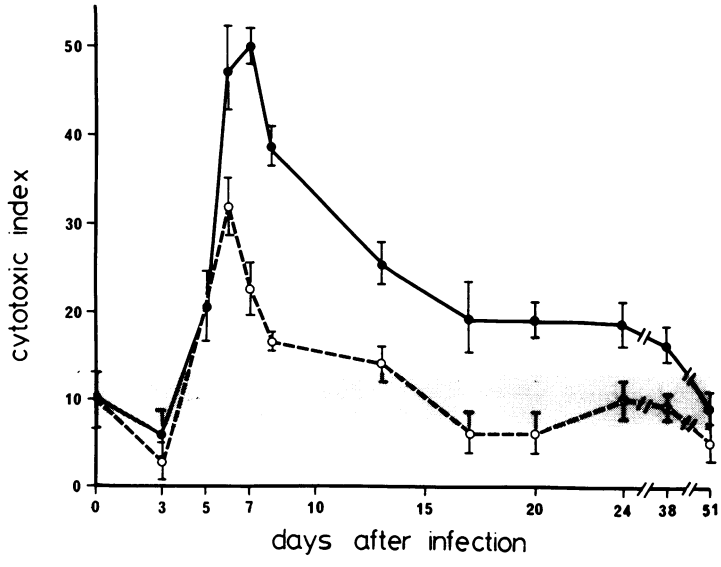

FIG. 3. *Cytotoxic activity (mean of 20 replicates ± 95% confidence limits) of spleen cells from mice at various intervals after infection with Sendai and Kunz viruses. Symbols: (●) Sendai-infected mice; (○) Kunz-infected mice. Shaded bar indicates limits of cytotoxic activity of spleen cells from uninfected mice.*

response 4 to 7 days after infection with Sendai virus coincides with the time during which the titer of virus present in the lungs is already declining rapidly (22). It has been shown in influenza infection in mice that a cell-mediated immune response can be demonstrated with cells from local lymph nodes before it is detectable with spleen cells (5). It is therefore possible that in Sendai-infected mice a local response might coincide even more closely with the eradication of virus from the lung than does the systemic response. However, the timing of the appearance of local antibody in the lung tissue (4, 22) indicates that antibody may also be related to inhibition of virus growth.

During the development of the assay, an unexpected finding was the ability of unsensitized spleen cells to kill Sendai virus-infected target cells. A similar phenomenon of "natural cytotoxicity" has recently been reported in both human lymphocytes (20) and mouse spleen cells (13, 16); however, the mechanisms responsible for this cell killing remain obscure and seem to vary between systems.

A cell-mediated cytotoxicity directed against Sendai-infected L cells, at levels intermediate between those observed with unsensitized and Sendai-sensitized mice, was found in the spleen cells of mice infected with the serologically distinct Kunz virus. This activity showed a similar course of development to that observed in Sendai-infected mice, coupled with a slightly more rapid rate of decline. This finding is at variance with the work of several other authors, who have reported no significant cross-reaction in the cell-mediated response to either totally nonrelated (6) or serologically related (5) viruses.

Evidence that indicates that virus-induced derepression of transplantation antigens may be a widespread phenomenon is accumulating (10, 11, 18, 19). Preliminary results in this laboratory using serological techniques for the detection of surface antigens indicate that Sendai virus infection of L cells may result in a similar derepression of H-2 antigens. The possibility that such a phenomenon occurs in vivo in both Kunz- and Sendai virus-infected animals must be considered. If this were so, infected mice would be simultaneously sensitized not only to viral antigens, but also to foreign tissue antigens. The relatively short-lived elevated cytotoxic activity observed in the spleen cells of Kunz-infected mice might therefore be due to, and directed against, derepressed transplantation antigens. It remains to be determined whether Sendai-infected mice show a similar elevated level of cytotoxic activity against Kunz virus-infected L cells.

Work is currently in progress to identify the cell types responsible for these various cytotoxic reactions, to determine the importance of such cell-mediated immune responses, and, particularly, to clarify the role of the T cell in the pathogenesis of acute respiratory viral infection.

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