

Effect of Cyclophosphamide on Infections in Mice Caused by Virulent and Avirulent Strains of Influenza Virus

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Induced immunosuppression with the drug cyclophosphamide was shown to convert the relatively harmless infection with an avirulent strain of Kunz influenza virus into a fatal pneumonic illness. The drug was also shown to increase the mortality of mice infected with low concentrations of a virulent variant of this strain, but it delayed the time of death of mice that were infected with high concentrations of the same variant. The probable roles of immune and inflammatory mechanisms in recovery from primary influenza virus infections are discussed.

Immunosuppressive regimens usually increase susceptibility to virus infection and also increase the severity of these infections. We have previously shown that the nonlethal infection of mice with Sendai virus can be converted into a fatal pneumonic illness when cyclophosphamide is administered (7). A similar effect of this drug has been seen with encephalomyocarditis (4), dengue, and West Nile viruses (2), and with *Mycoplasma pneumoniae* (8).

There are reports in the literature which suggest that immunosuppression does not exert a deleterious effect on experimental influenza infections. Indeed, it has been shown that cyclophosphamide delays the mortality of PR8-infected mice (9), and a similar sparing effect of X-irradiation on CAM influenza virus infection of the same animal has been noted (1). Antilymphoid sera have also been reported to have no effect on influenza virus infection of mice (3).

With a view to exploring this apparently anomalous behavior of influenza virus in immunosuppressed hosts, we have studied the effect of cyclophosphamide on infections in mice caused by virulent and avirulent strains of Kunz influenza virus.

MATERIALS AND METHODS

Virus. Virulent and avirulent strains of the Kunz subtype A1 virus were used. The virulent strain was obtained by serial passage in the lungs of mice as previously described (5). Both viruses were propagated in embryonated hen eggs to prepare pools for mouse inoculation and were assayed for infectivity in monkey kidney tissue culture.

Infection of mice and cyclophosphamide treatment. Five-week-old CD1 mice were lightly anaesthetized with ether and then inoculated intrana-

sally with 0.1 ml of virus suspension containing the stated concentration of virus.

A dose of 300 mg of cyclophosphamide per kg was administered subcutaneously to the mice on the day before infection, and a dose of 100 mg/kg was administered by the same route on the 5th day after infection.

Experimental procedure. The seed pools were diluted in broth saline so that each contained exactly 10^6 mean tissue culture doses (TCD₅₀)/ml. Serial dilutions were then made providing inocula ranging in concentration from 10^8 to 10 TCD₅₀/ml. Each of these dilutions was inoculated into a batch of 10 mice that were treated with cyclophosphamide and also into an equal number of untreated mice that served as controls.

After inoculation, the mice were observed daily for 14 days, and deaths associated with typical pneumonitis were recorded. Mice surviving to the end of the observation period were exsanguinated, and levels of specific antibodies in their sera were determined by hemagglutination inhibition tests.

The immunosuppressant effectiveness of this cyclophosphamide regimen was demonstrated by observing the development of antibodies in the treated and untreated groups of mice infected with suspensions containing 10^8 TCD₅₀ of both the virulent and avirulent viruses per ml. The mice infected with the avirulent strain first developed antibodies on day 6 after infection, and titers had risen to 512 by day 14. Mice infected with the virulent strain showed a slightly earlier response, with antibody appearing for the first time on day 5. The titers rose rapidly, but prolonged observation was impossible because of the high mortality at this time. This restriction also applied to the mice that were infected with both viruses and treated with cyclophosphamide, but none of these developed antibodies at any time during the first week of infection.

RESULTS

The effect of cyclophosphamide on the mortality of mice infected with the avirulent and

virulent strains of Kunz virus is shown in Fig. 1 and 2.

Avirulent strain. The original egg-adapted strain of the virus was relatively harmless for mice. From Fig. 1 it can be seen that no deaths occurred in the mice infected with suspensions containing concentrations of virus which ranged for 10 to $10^{2.5}$ TCD₅₀/ml. A few mice that had been inoculated with suspensions containing 10^3 , 10^4 , and 10^5 TCD₅₀ of virus per ml succumbed, but the mortality never exceeded 30%. These results are in agreement with previous findings with this virus (5).

Infection with this virus was dramatically altered by cyclophosphamide treatment. Some deaths were noted in all groups infected, with the final mortality ranging from 30% in the group infected with the suspension containing 10 TCD₅₀/ml to 90% in those infected with the suspension containing 10^5 TCD₅₀/ml.

The effect of cyclophosphamide on the avirulent Kunz virus infection appeared to be exactly the same as its effect on mice infected with Sendai virus (7) in that it converted a generally harmless infection into a fatal pneumonic illness.

Virulent strain. It had previously been shown that serial passage in mouse lung appreciably increases the virulence of the Kunz strain of influenza virus for mice (5). In these experiments the mortality after inoculation with suspensions containing concentrations of virus ranging from 10 to 10^5 TCD₅₀/ml ranged from 10 to 100% (Fig. 2).

Cyclophosphamide was shown to have two quite distinct effects on the infections caused by this virulent strain. It can be seen from Fig. 2 that, when low concentrations of virus (10 to 10^3 TCD₅₀/ml) were used, cyclophosphamide treatment appeared to enhance the virulence of the

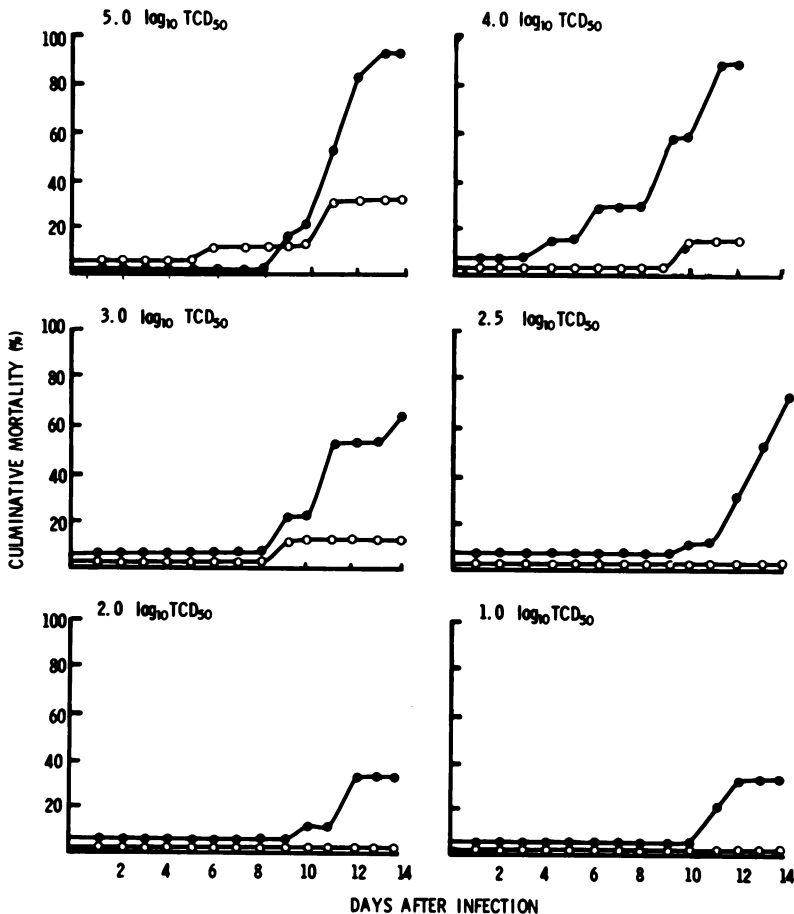


FIG. 1. Effect of cyclophosphamide on the mortality of mice infected with the avirulent variant of Kunz influenza virus. Symbols: ●, treated with cyclophosphamide; ○, untreated.

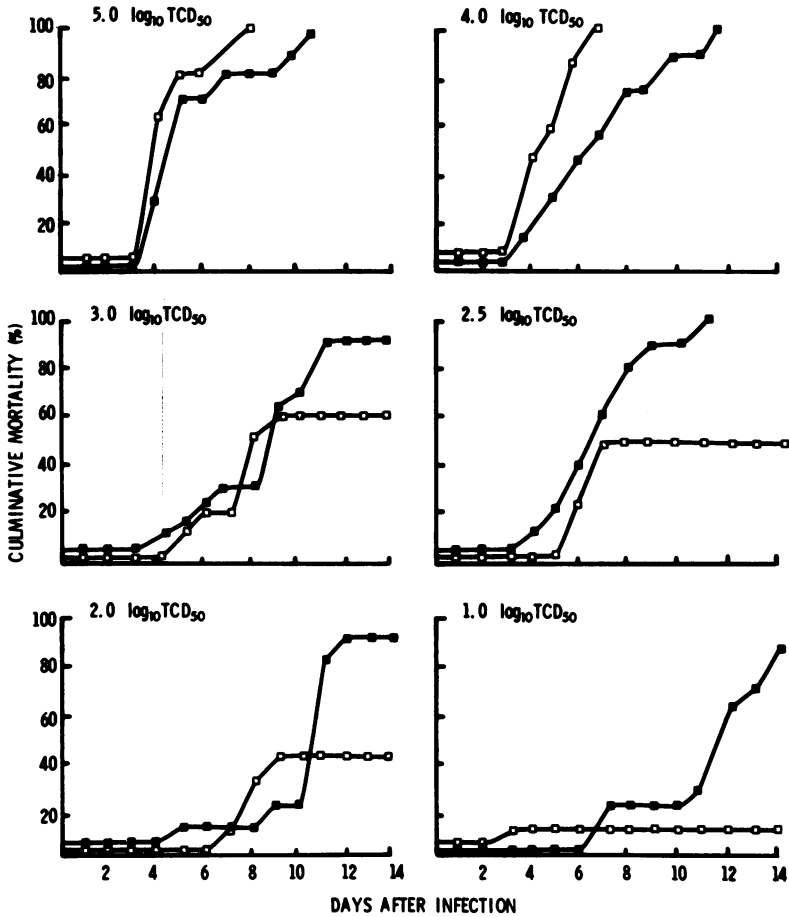


FIG. 2. Effect of cyclophosphamide on the mortality of mice infected with the virulent variant of Kunz influenza virus. Symbols: ■, treated with cyclophosphamide; □, untreated.

virus. Virtually all the mice inoculated with these low concentrations of virus and treated with cyclophosphamide died, whereas the mortality of the untreated controls ranged from only 10 to 60%. The effect of cyclophosphamide on infections induced by low concentrations of this virus is essentially similar to its effect on the avirulent virus infections that have been described above.

An entirely different effect was obtained when mice infected with suspensions containing high concentrations (10^4 and 10^5 $\text{TCD}_{50}/\text{ml}$) were immunosuppressed. It can be seen from Fig. 2 that all the untreated mice died within 1 week after being inoculated with these high doses of virus. Cyclophosphamide was shown to have a sparing effect on these high virulent infections, for although all the treated mice eventually died the final deaths did not occur until the second week after inoculation. The results

obtained with these high concentrations of the virulent Kunz strain are similar to those obtained by Sing et al. with PR8 influenza virus (9).

DISCUSSION

Recovery from respiratory viral infections is dependent on the host rapidly recruiting competent immunocytes in the submucosa of affected areas (5, 6). Prevention of this local response by powerful immunosuppressant drugs such as cyclophosphamide results in failure of viral eradication and more serious and frequently fatal illness (7).

In general, these experiments have shown that cyclophosphamide exerts a similar effect on experimental influenza infections. When mice infected with the avirulent strain of Kunz virus were treated with this drug, the usually harmless infection was converted into a fatal

pneumonic disease. Even mice infected with low doses of the virulent variant of this strain had higher mortality rates when treated with cyclophosphamide. The apparent exception to this rule was the finding that cyclophosphamide significantly delayed the death of mice infected with high doses of the virulent strain.

It would appear therefore that recovery from influenza infections is equally as dependent on the efficient mounting of the immune response as are the infections caused by Sendai virus (6), encephalomyocarditis virus (4), and togaviruses (2). Previous studies have suggested that the fatal infection induced by the virulent Kunz strain was due to its newly acquired ability to extensively infect and damage alveolar cells and that the resulting inflammatory changes are responsible for the pneumonic termination of the illness (5). These events take place in spite of the development of intense immune response in the alveolar regions, which is evident from the appearance of large numbers of antibody-containing cells at this site. Since the immune response is incapable of restraining extensive infection of the alveoli, it is hardly surprising that immunosuppression can have little further deleterious effect on such infections. This interpretation would apply to the infection caused by high doses of the virulent Kunz strain described here and to similar infections by the PR8 (3, 9) and CAM (1) strains of influenza. The beneficial effect of cyclophosphamide on mice infected with high doses of the virulent Kunz strain and the PR8 strain (9) is pre-

sumably due the anti-inflammatory properties of the drug.

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