STEPHEN

FIRST SUCCESSFUL USE OF A CHEMICAL COMPOUND FOR THE PROPHYLAXIS AND TREATMENT OF A LETHAL, SYSTEMIC, VIRAL INFECTION COMMON TO MAN AND SUBHUMAN PRIMATES (U)

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Virus diseases remain the most important uncontrolled health problem confronting both civilian and military populations. They produce enormous worldwide economic losses through death and incapacitation. Though most virus diseases are incapacitating and self-limiting, some result in death. Loosli (1) estimated that man suffers seven years of virus-induced illness during an average 70-year life span. While vaccines have proven highly effective in certain instances, immunization methods are not available for all virus diseases. The reasons for this can be summarized as follows: (1) Many viruses are not easily adapted to the production of safe, effective vaccines because of poor antigenicity or high virulence. (2) Vaccines are usually virus-specific, and therefore are only effective for a single disease. (3) Vaccines are not usually effective after infection has occurred. (4) Certain viruses (notably influenza virus) change antigenically so that vaccines soon lose their effect. (5) Certain populations of people, for religious or other reasons, cannot be immunized with existing vaccines.

Even though we have a safe, effective vaccine for use against the virus of yellow fever, epidemics have occurred recently in unvaccinated people in Nigeria. The virus is endemic in many tropical and subtropical areas of the world. For this reason, and because laboratory studies *in vitro* suggested that yellow fever virus was susceptible to the antiviral activity induced by interferon, we selected yellow fever virus infection of monkeys to evaluate the *in vivo* antiviral activity of antiviral drugs.

Drugs are necessary for use in the prophylaxis and treatment of virus diseases for which vaccines have yet to be evaluated or proven effective. With antiviral drugs, potential exists for broad-

spectrum activity. This attribute is especially desirable, since rapid diagnosis of virus diseases is not, as yet, possible.

Since the discovery of interferon by Isaacs and Lindenmann in 1957 (2), much attention has been focused on the synthesis of interferon and, more recently, on the induction of endogenous interferon. The interferon system is now known to be important in the host defense mechanism, both early in infection because of induction of antiviral protein and late in infection because of its possible role in potentiating humoral or cell-mediated immune responses. Interferon, whether induced exogenously or endogenously, has a broad spectrum of antiviral activity. Theoretical considerations initially indicated that poly I·poly C might play a key role in the therapy of viral diseases through induction and release of endogenous interferon. However, poly I·poly C in man, subhuman primates, and certain other animals, is rapidly degraded enzymatically to an inactive state. Poly I·poly C stabilized with low-molecular-weight (2,000) poly-l-lysine and carboxymethylcellulose (PIC-L) is 5 to 10 times more resistant to hydrolysis by pancreatic ribonuclease and human serum than the unstabilized poly I·poly C (3). Levy et al. (3) reported serum interferon levels as high at 6,000 units/ml in rhesus monkeys following intravenous injection of 3.0 mg/kg of the complex.

Since peak interferon levels were expected to occur 8 hr after the PIC-L was given, we injected the first 3.0 mg/kg dose of PIC-L 8 hr prior to virus challenge. Groups of monkeys were also treated initially at 8 and 24 hr after virus inoculation. Additional injections of PIC-L were made on days 1 to 4, 7, 9, 11, 15 and 17. The monkeys were bled daily for viremia, antibody, and interferon assays, and were challenged subcutaneously with 1,000 plaque-forming units (PFU) of the Asibi strain of yellow fever virus. Previous experiments have shown that this dose of virus was uniformly lethal between 4 and 6 days after infection.

Untreated virus control monkeys in the present experiments all died (Table I). When treatment was initiated at -8 or +8 hr after virus inoculation, either 71 or 75% of the monkeys survived. None of the monkeys receiving treatment at +24 hr survived.

The viremia produced in untreated, infected control monkeys is compared with that of monkeys given the first dose of PIC-L at -8 hr, +8 hr, or +24 hr after virus inoculation (Fig. 1). The geometric mean virus concentration of each group of monkeys was plotted against days after virus inoculation. Untreated control monkeys, shown by the open circles, were detectably viremic by day 2 and reached peak viremia by day 4, at which time some deaths occurred. All of the untreated control monkeys died by day 5.

Recall that monkeys received the initial dose of PIC-L at different times. The group treated initially 8 hr prior to virus in-

TABLE I. RESPONSE TO YELLOW FEVER VIRUS INFECTION OF MONKEYS TREATED WITH PIC-L

Time of Initial Treatment	Dead/Total	% Survival	Mean Time to Death, Days ± SEM
Untreated*	5/5	0	4.8 ± 0.4
-8 hr*	2/7	71	11.0 ± 1.0
+8 hr	1/4	75	14
+24 hr	2/2	0	6 ± 0
PIC-L only	0/4	100	--

*Composite data from two experiments.

oculation is denoted by triangles (Fig. 1). Monkeys treated initially at 8 and 24 hr after virus inoculation are shown by diamonds and squares, respectively. The onset of viremia was delayed from 3 to 7 days for th monkeys treated initially 8 hr prior to virus inoculation. In addition, the peak viremia of these monkeys was at least 1,000- to 10,000-fold lower than that of the untreated virus control monkeys. In previous studies with yellow fever virus disease in rhesus monkeys, all monkeys developing detectable viremia subsequently died, thus making the present observations even more exciting. Five of seven monkeys in this group survived, and all of the survivors developed serum-neutralizing antibody by day 17 after virus inoculation. On day 37 postinfection, when the antibody titer was greater than 1:320, the monkeys were challenged with 1,000 PFU of Asibi strain yellow fever virus. No viremia or mortality was detected following the second challenge.

Two of four monkeys treated with PIC-L 8 hr after infection were not detectably viremic and are not presented in Figure 1. The peak titer of the viremic monkeys was lower than that of control monkeys, but no delay in onset of viremia was observed. For monkeys initially treated 24 hr after infection, the time to onset of viremia was 24 hr earlier than for untreated control monkeys, and the time to death was longer. Both monkeys died. The peak virus titer did not differ from control values, but the peak was delayed 24 hr.

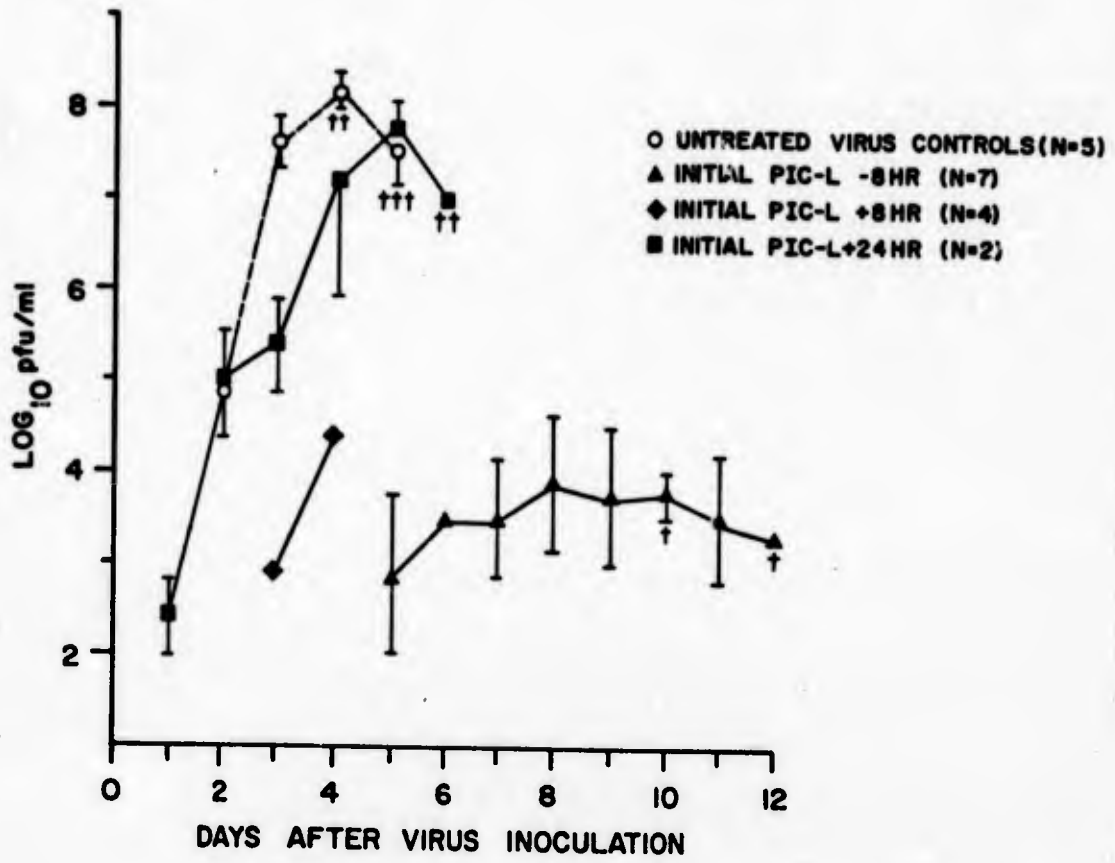

Fig. 1. Effect of multiple doses of PIC-L on serum virus recovery (Log_{10} PFU/ml) in monkeys challenged with Asibi strain yellow fever virus. PIC-L was given 8 hr prior to virus inoculation and on days 1 to 4, 7, 9, 11, 15 and 17. (O) Virus recovery from untreated virus control monkeys. (▲) Virus recovery from monkeys given PIC-L initially 8 hr prior to inoculation of virus. (◆) Virus recovery from monkeys treated with PIC-L 8 hr after virus inoculation. (■) Virus recovery from monkeys in which initial therapy was delayed 24 hr.

Two of four monkeys given PIC-L 8 hr prior to infection, shown by the daggers, died either on day 10 or 12 after infection. One of four monkeys given the complex initially 8 hr after infection died on day 14 postinfection. Two of these monkeys were not viremic prior to death. It is possible that these monkeys did not die as a direct consequence of the yellow fever virus infection, since untreated monkeys always have high viremias prior to death. Monkeys given 6.0 mg/kg of PIC-L daily for 10 days did not die.

The antibody responses of the surviving monkeys are shown as Figure 2. Recall that we have previously stated that monkeys given PIC-L initially 8 hr after virus inoculation had viremia earlier than monkeys treated initially 8 hr before virus inoculation. This time difference in initiation of viremia could account for the apparently earlier antibody response of monkeys first treated 8 hr after virus inoculation. There were no differences between antibody titers of the two groups of surviving monkeys by day 42. It is important to note that successful treatment of yellow fever virus disease in rhesus monkeys was accompanied by the development of a strong humoral antibody response that rendered the monkeys resistant to subsequent virus challenge. This finding was particularly interesting, since many potentially useful antiviral agents do not permit sufficient antigenic stimulation of host defense mechanisms to render the host resistant to subsequent infection. Untreated monkeys, and monkeys treated initially 24 hr after virus inoculation, did not survive long enough to develop detectable serum-neutralizing antibody.

The serum interferon responses of monkeys treated at various times are shown in Figure 3. It is interesting to note that interferon was detected in the serum of the control monkeys beginning on day 3 after virus inoculation, with a peak greater than any of the treated monkeys.

The interferon response of monkeys treated initially 8 hr before or 8 hr after virus inoculation was similar to that of the uninfected monkeys given only PIC-L. It is possible that a threshold concentration of virus must be achieved to stimulate production of interferon in the infected animal, since the maximum viremia of monkeys in the groups treated 8 hr before or after virus inoculation was at least 1,000- to 10,000-fold lower than that of untreated virus control monkeys, or monkeys treated initially at 24 hr.

Since all of the untreated virus control monkeys died in spite of high serum concentrations of interferon, and some treated monkeys survived, one can assume that certain factors are important determinants of successful prophylaxis and treatment. Time of initial interferon stimulation is probably the most important factor. This is emphasized by the observation that treatment at -8 hr or +8 hr was successful, whereas treatment at 24 hr was not. These data

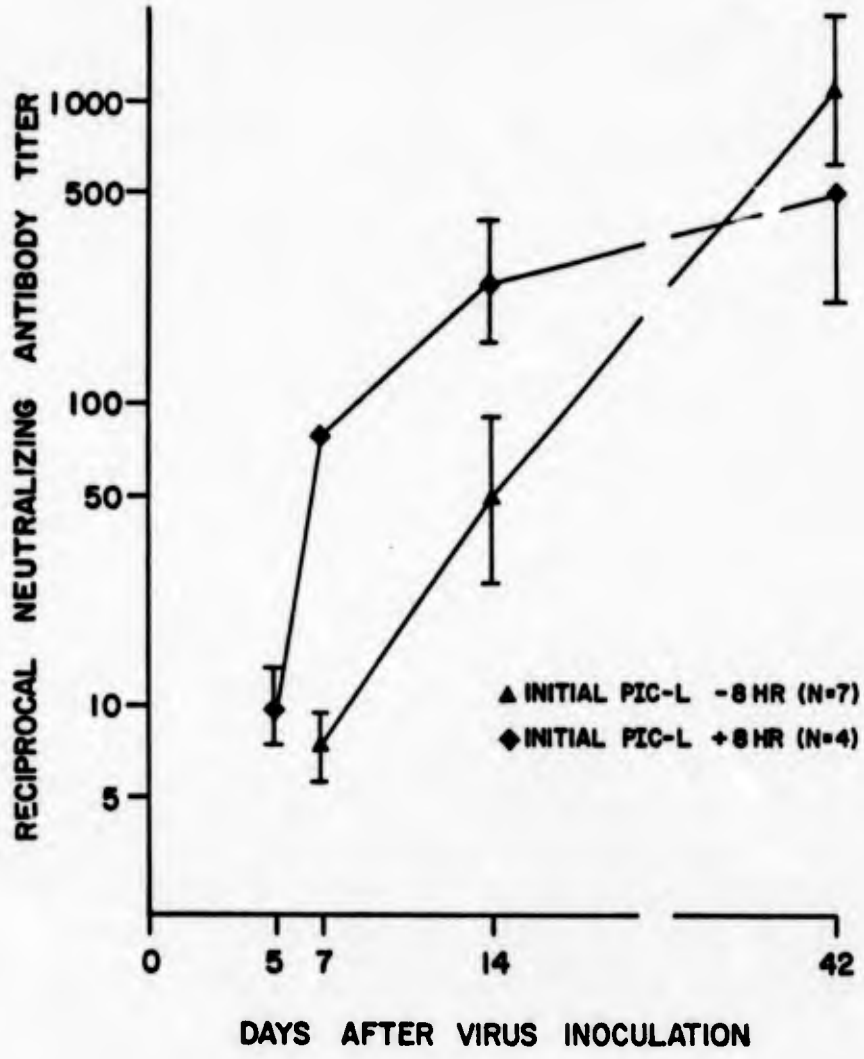

Fig. 2. Serum-neutralizing antibody titer (PR_{80}) of monkeys given PIC-L and challenged with Asibi strain yellow fever virus. PIC-L was given on days 0 to 4, 7, 9, 11, 15 and 17. (▲) Antibody titer of monkeys given PIC-L initially 8 hr prior to inoculation of virus. (◆) Antibody titer of monkeys receiving the first dose of PIC-L 8 hr after virus inoculation.

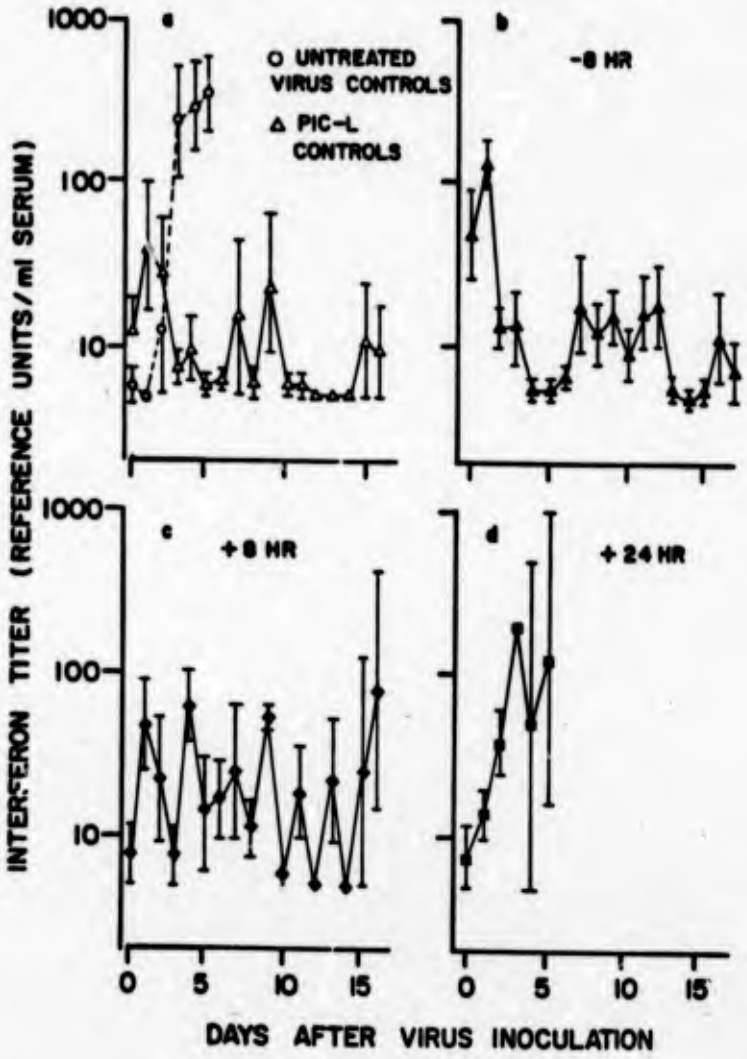

Fig. 3. Relationship of the serum interferon response of monkeys challenged with Asibi strain yellow fever virus to time of initial treatment with PIC-L. (a) Serum interferon response of uninfected PIC-L control monkeys given PIC-L on days 0 to 4, 7, 9, 11, 15 and 17 (Δ). Serum interferon response of untreated virus control monkeys (O). (b) Serum interferon response of monkeys given PIC-L 8 hr prior to virus challenge and on days 1 to 4, 7, 9, 11, 15 and 17. (c) Interferon response of monkeys receiving intial PIC-L treatment 8 hr after virus inoculation and on days 1 to 4, 7, 9, 11, 15 and 17. (d) Interferon response of monkeys receiving delayed treatment 24 hr after challenge and on days 2 to 4, 7, 9, 11, 15 and 17.

appear to limit severely the therapeutic role of PIC-L. While this observation is ostensibly true, one should remember that yellow fever virus disease is not uniformly lethal in man. Recent unpublished correlations between *in vitro* and *in vivo* titer data have clearly shown that 1,000 PFU of yellow fever virus were greater than or equal to 10,000 median lethal doses of the virus. Since the challenge dose of virus is a critical determinant of antiviral activity, it is possible that reducing the challenge dose of virus might increase the therapeutic efficacy of PIC-L.

As indicated earlier, most virus diseases are self-limiting. In the case of virus diseases caused by interferon-sensitive viruses, the injection of PIC-L might enhance the rate of recovery. Enhanced recovery might be induced directly by stimulating the production of antiviral protein, the humoral antibody system, or cell-mediated immunity.

CONCLUSIONS

PIC-L is effective for the prophylaxis and early treatment of yellow fever virus infection of monkeys. Its importance is emphasized by the potentially overwhelming challenge dose used in the present studies.

The demonstrated efficacy of this new interferon inducer should prove valuable for the control of other virus diseases caused by interferon-sensitive viruses for which no effective vaccines exist.

PIC-L does not completely inhibit virus replication, and therefore possesses an important attribute of an ideal antiviral drug--it is not immunosuppressive.

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In conducting the research described in this report, the investigators adhered to the "Guide for the Care and Use of Laboratory Animals," as promulgated by the Committee on Revision of the Guide for Laboratory Animal Facilities and Care of the Institute of Laboratory Animal Resources, National Research Council. The facilities are fully accredited by the American Association of Accreditation of Laboratory Animal Care.