THERAPY OF INFECTION WITH PNEUMONIA VIRUS OF MICE (PVM)

Effect of a Polysaccharide on the Multiplication Cycles of the Virus and on the Course of the Viral Pneumonia

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Inhibition of multiplication of pneumonia virus of mice (PVM) and mumps virus by the type specific capsular polysaccharides of Friedländer bacilli has been reported in previous papers (1, 2). Not only does the polysaccharide inhibit viral multiplication when given as long as 4 days after inoculation of either virus, but also significant inhibition is obtained with only a few micrograms of the purified material (1, 2). The available evidence indicates that the polysaccharide does not act directly upon either virus to cause inactivation nor does the substance combine with either agent (1–3). Also, it appears that release of PVM or mumps virus from infected host cells is not prevented by the complex carbohydrate (4). These findings have led to the hypothesis that the polysaccharide acts upon the susceptible host cell to combine with, or compete for, some cell constituent which is present in limited quantity, not required to maintain life of the cell, but necessary for viral multiplication.

If this postulate were valid, it would be anticipated that two verifiable predictions should follow: (1) Inasmuch as both PVM and mumps virus appear to multiply in discrete cycles (4, 5), the polysaccharide should inhibit the incremental increase in viral concentration when given during the latent period of the cycle; (2) The polysaccharide should limit viral multiplication after infection is well established but submaximal, and as a result (6) inhibit progress of the pathological lesion (7). In order to test these predictions, PVM was selected as the agent of choice because: (1) it produces fatal viral pneumonia in its *natural host*; (2) the qualitative and quantitative aspects of infection with the agent have been studied extensively (6, 8, 9); (3) the temporal limits of the latent period of a single cycle of multiplication with the virus have been determined (5).

It is the purpose of this paper to show that the capsular polysaccharide of Friedländer bacillus, type B, not only inhibits the multiplication of PVM when given as long as 10 hours after a large inoculum of virus, but also modifies the course of the severe viral pneumonia induced in mice so that the majority of treated animals recover completely.

Materials and Methods

Virus.—The strains of PVM employed, the method of passage, and means of storage were identical with those described in the preceding papers (5, 6).

Mice.—Albino Swiss mice of the Rockefeller Institute strain as well as similar strains from commercial breeders were employed. As a routine, each experimental group contained 6 mice, 3 to 4 weeks of age.

Hemagglutination Titrations.—Suspensions of infected mouse lungs were prepared either in 0.85 per cent NaCl buffered at pH 7.2 with 0.01 M phosphate buffer or in distilled water. The titrations were performed in a manner identical with those previously described (5, 8).

Infectivity Titrations.—These were carried out as in previous studies (10).

Polysaccharide Preparations.—The capsular polysaccharide of Friedländer bacillus, type B (Fr.B) was obtained from Dr. Walther F. Goebel, the Rockefeller Institute, New York. Solutions of polysaccharide were prepared as previously described (2) and sterilized by heating at either 70°C. for 30 minutes or 100°C. for 2 minutes.

Immune Mouse Serum.—Mice were inoculated intranasally with a sublethal dose of PVM, less than 1.0 M.S.50, and in 2 weeks were injected intraperitoneally with 0.5 cc. of a 10 per cent infected lung suspension containing about 10^4 M.S.50 doses. The animals were bled from the heart 2 weeks later. Serum was stored without preservative at 4°C.

EXPERIMENTAL

Effect of Capsular Polysaccharide of Friedländer Bacillus, Type B, on First Cycle of Multiplication of PVM.—If the multiplication of PVM is inhibited by Fr.B. as a result of some effect within the host cell as has been postulated (1), the polysaccharide should be active during the latent period of a single cycle of multiplication of the virus. As shown in the preceding paper (5), the latent period with PVM is of about 15 hours' duration. Experiments were carried out to determine if viral multiplication could be inhibited by injection of Fr.B at various times during the latent period, and whether there was a critical time after which the substance would have no significant effect upon the formation of new viral particles during a single multiplication cycle.

Mice were inoculated intranasally with $10^{3.5}$ M.S.50 doses of PVM, and various groups were each given a single intranasal injection of Fr.B, 0.1 mg. per mouse, at 2, 4, 8, 10, or 12 hours afterwards. For controls, other infected mice were given saline, 0.05 cc. intranasally, at corresponding periods. Groups of mice from each series were killed at 4- to 8-hour intervals commencing 20 hours after inoculation of PVM. The lungs were stored at −28°C. until the completion of the experiment after which suspensions were made in distilled water and hemagglutination titrations carried out with 0.05 per cent mouse RBC as described previously (5).

The mean results of three separate experiments are presented graphically in Fig. 1. Intranasal injection of Fr.B as long as 4 hours after initiation of infection with a large inoculum of PVM completely inhibited viral multiplication as determined by the hemagglutination technique. Even when polysaccharide was injected as late as 10 hours after viral inoculation, there was significant inhibition of multiplication. However, when the inoculation preceded the injection of Fr.B by 12 hours, there was no definite decrease in the quantity of

virus formed during a single cycle of multiplication although there was a consistent retardation of the incremental period. That there was a progressive decrease in the inhibitory action of the polysaccharide from 4 to 12 hours during the latent period may be explained on either of two bases: (1) that the time required for Fr.B to reach all infected cells and to produce its effect is prolonged; or (2) that viral particles are formed at slightly different rates and therefore that some particles reach a stage of development at which Fr.B is ineffective before other particles.

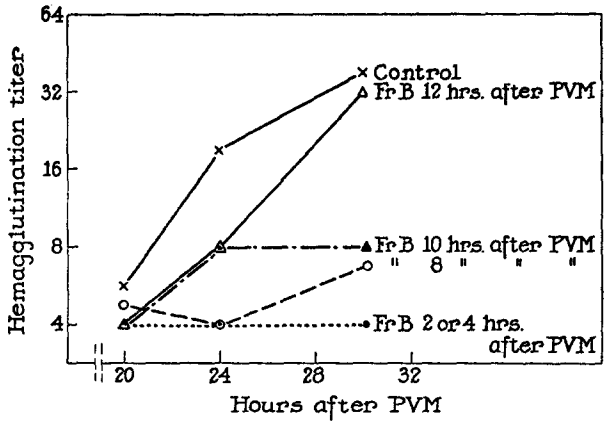

FIG. 1. Inhibition of multiplication of PVM in the mouse lung during the latent period of the first cycle of multiplication. A single injection of 0.1 mg. of Fr.B per mouse was given intranasally at the indicated time after inoculation of $10^{3.5}$ M.S.50 doses of virus.

Effect of Fr.B Injected at End of Latent Period.—That the polysaccharide did not markedly alter the first cycle of multiplication of PVM when it was injected 12 hours after a large inoculum was demonstrated above (*cf.* Fig. 1). Inasmuch as earlier work (1) indicated that inhibition could be obtained late, *i.e.*, 4th day, in the infectious process, it seemed probable that when polysaccharide was given late enough in the first cycle to be ineffective it would cause inhibition of the next cycle of multiplication.

Fr.B, 0.1 mg. per mouse, was injected intranasally 18 hours after inoculation with $10^{3.8}$ M.S.50 doses of PVM, and the viral concentration in the mouse lungs was then determined at frequent intervals. Except for the time of polysaccharide injection, and differences in the periods at which groups of mice were killed, these experiments were carried out in a manner similar to those described in the preceding section.

The results of these experiments are presented in Fig. 2. As was to be expected, Fr.B injected 18 hours after PVM did not demonstrably alter the first cycle of viral multiplication, but its effect on the ensuing cycle was marked. It should be emphasized that the latent period with this virus is of the order

of 15 hours (5) and in terms of the hypothesis under consideration it was not to be anticipated that the polysaccharide would inhibit multiplication during a single cycle when given after completion of the latent period. Not only was multiplication during the subsequent cycle inhibited, but also a definite decrease in viral concentration occurred during this time period. As was demonstrated in the preceding paper (5), when PVM is prevented from multiplying, the concentration of the agent progressively decreases in the mouse lung.

The evidence presented in Figs. 1 and 2 indicates that during the first two-thirds of the latent period, multiplication of PVM is inhibited by a single

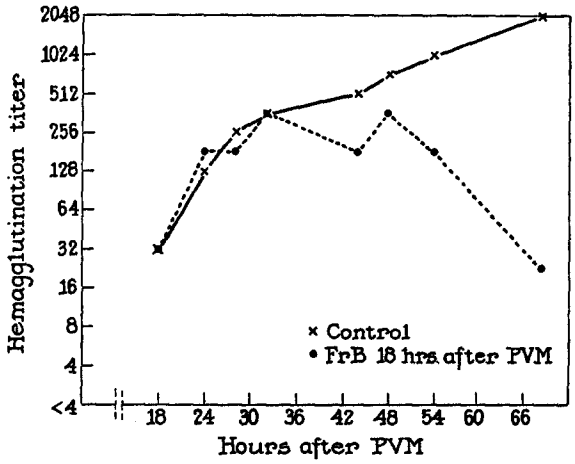

FIG. 2. Inhibition of multiplication of PVM in the mouse lung during the second cycle of multiplication. A single injection of 0.1 mg. of Fr.B per mouse was given intranasally 18 hours after inoculation of $10^{3.8}$ M.S.50 doses of virus.

injection of 0.1 mg. of Fr.B. Thereafter, no significant inhibition is obtained during the initial cycle. Giving the polysaccharide after the completion of the latent period of the first cycle of multiplication results in its being present before the next cycle commences. As a consequence, multiplication of the virus during the second cycle is inhibited.

Effect of Fr.B on the Mouse Lung.—While carrying out the experiments described in this report, it was found that the intranasal injection of Fr.B in amounts of 0.1 mg. or more per mouse often resulted in the development of some pulmonary lesions demonstrable 1 to 2 days later. The lesions were irregular in distribution and size, seldom involved more than 30 per cent of the lung, and reached maximal size 4 days after the injection. Disappearance of the lesions occurred rapidly and by the 6th day almost all had resolved completely. Microscopic examination showed the lesions to consist mainly of areas of atelectasis although in the gross they were indistinguishable from areas of

pneumonic consolidation. It seemed of some importance to determine the relationship of such lesions to the capacity of the polysaccharide to inhibit multiplication of PVM in the mouse lung.

It was found that the capacity to produce pulmonary lesions was directly related to the viscosity of the polysaccharide solutions. As is shown in Fig. 3 when 0.02 mg. of Fr.B was injected intranasally, no lesions were produced in any animal, and yet this small quantity of polysaccharide inhibited multiplication of PVM markedly. The same preparation of Fr.B was oxidized with 0.03 M periodic acid at pH 5.0 for 160 minutes and 24 hours, respectively, as previously described (2).[1] As is demonstrated in Fig. 3, 0.1 mg. of Fr.B which had been oxidized for 160 minutes produced definite lesions in the lung, whereas

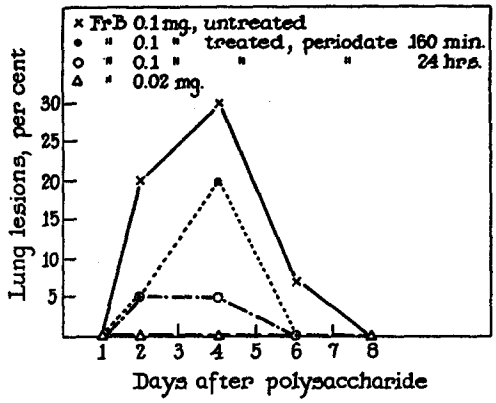

FIG. 3. Extent and duration of lesions caused in the mouse lung by intranasal injection of different quantities of Fr.B before and after treatment with 0.03 M HIO_4 at pH 5.0.

a similar quantity oxidized for 24 hours produced only equivocal lesions in 50 per cent of the injected mice. Despite the fact that Fr.B treated with periodate caused only minimal lesions, it retained in undiminished degree its ability to inhibit multiplication of PVM. Similar results were obtained previously (1). These findings indicate that the production of transient pulmonary lesions by certain preparations of Fr.B is not directly related to inhibition of viral multiplication. In this connection it has been demonstrated previously (2) that large quantities of Fr.B produce no demonstrable lesions in the chick embryo or its chorioallantoic membrane and yet the multiplication of mumps virus is inhibited in this host species by the polysaccharide.

Modification of the Course of PVM Pneumonia by Fr.B.—The evidence presented in a preceding paper (6) indicates that the extent of the pathological

[1] The oxidation procedures were kindly carried out by Dr. Walther F. Goebel, the Rockefeller Institute, New York.

lesion produced in the mouse lung is directly related to the concentration of PVM present at a given time. It appears, from the data given above, that multiplication of the virus can be interrupted by Fr.B in the first cycle if the polysaccharide is injected during the first two-thirds of the latent period. In addition, subsequent cycles of multiplication of the agent are inhibited by the substance (1). These findings made it seem probable that reduction in the quantity of PVM formed as a result of inhibition of multiplication by Fr.B would also reduce the extent of the pneumonia which developed. It was thought that if the development of the pathological lesion were sufficiently impeded the animals would recover from the infection. In order to test this possibility, the following experiments were carried out:—

Numerous mice were inoculated intranasally with 10^2 M.S.50 doses of PVM. One large group of inoculated mice was given Fr.B, 0.02 mg. per mouse, intranasally 2 days after PVM inoculation and another group was treated similarly 3 days following the virus. Other groups of inoculated animals were injected with saline intranasally 2 and 3 days, respectively, following the virus. Groups of 6 mice in each series were killed at 2-day intervals beginning 4 days after infection. The lung lesions were scored as described previously (10), and the lungs were removed and stored at −28°C. until the completion of the experimental period. Hemagglutination titrations and, in certain instances, infectivity titrations were carried out on 10 per cent lung suspensions prepared in saline. Hemagglutination titers were determined with a final concentration of 0.4 per cent mouse RBC; because of this and the fact that the suspensions were prepared in saline, the titers are 16-fold lower than those for equivalent viral concentration determined by the modified procedure described in the preceding paper (5). The experimental period for each group was determined from the mean survival time.

The results of a typical experiment are shown in Fig. 4. The results obtained in the two control series, those given saline 2 and 3 days, respectively, following PVM, were almost identical and are presented together as mean values in both the upper and lower portions of the graph. The disease was uniformly fatal in the two control series; all mice had died by the 7th day at which time complete consolidation of the pulmonary tissue, *i.e.*, 100 per cent, had occurred. Mice which received a single intranasal injection of 0.02 mg. of Fr.B 2 days after inoculation with PVM showed a much less extensive disease. At the time of greatest pulmonary involvement, *i.e.*, 6 days, only 43 per cent, on the average, of the lung was consolidated as a result of the viral infection. Evidence of resolution was apparent at 10 days, at 16 days only small areas of gray consolidation remained, and all animals survived the experimental period. When 0.02 mg. of Fr.B was given as long as 3 days after inoculation, the course of the pneumonic process also was modified markedly. The pulmonary lesions were somewhat more extensive than in mice treated earlier in the disease, but on the average the largest amount of lung tissue which showed consolidation was only 57 per cent, and 66 per cent of the animals survived the experimental period.

In the lower portion of Fig. 4 is shown the concentration of virus in the same lungs as measured by hemagglutination titration. The curve of increase in the quantity of PVM in the lungs of control mice closely approximated that observed in earlier studies (6, 8); maximal concentration was reached 6 days

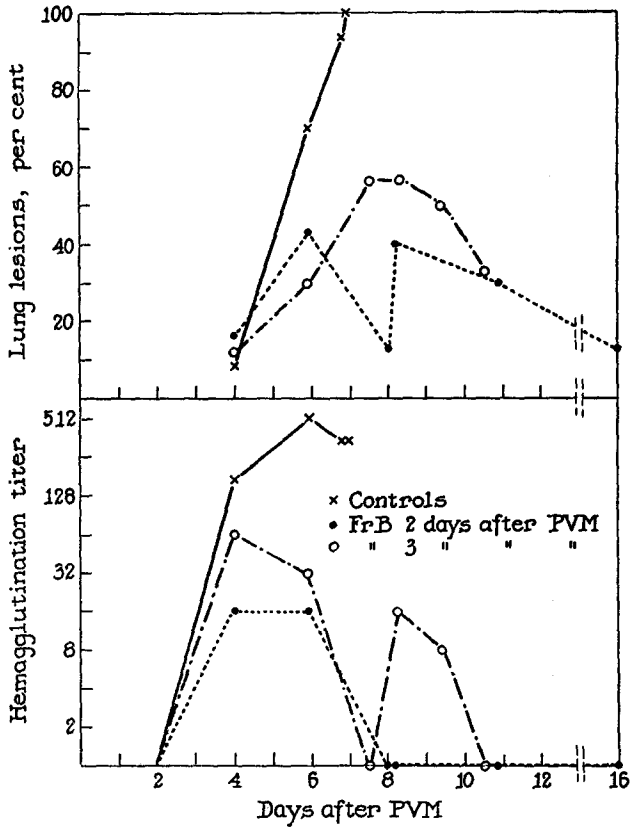

FIG. 4. Relation between the degree of inhibition of multiplication of PVM in the mouse lung and the extent of restriction in the amount of pneumonia. A single injection of 0.02 mg. of Fr.B per mouse was given intranasally either 2 or 3 days after inoculation with 10^2 M.S.50 doses of virus. Lung lesions and viral concentration were determined, in each instance in the same animals. All control mice died by the 7th day.

after inoculation of the virus. The increase in viral concentration in the lungs of mice that had received Fr.B either 2 or 3 days following inoculation was markedly restricted and it appears that not more than one cycle of multiplication was completed after the polysaccharide was given. After the 6th day the concentration of PVM in the lungs began to decrease and after 10 days no virus was demonstrable. Infectivity titrations with lung suspensions from

selected groups of mice in this experiment confirmed the results obtained with the hemagglutination technique and showed that there was a quantitatively similar difference in the amount of infective virus in the lungs of Fr.B treated animals as compared to those of controls.

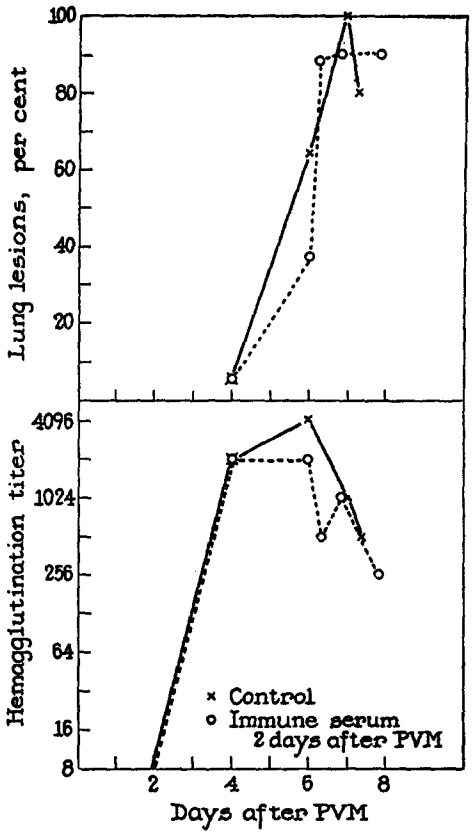

FIG. 5. Lack of effect of PVM immune mouse serum on the multiplication of PVM in the mouse lung and on the development of pneumonia when serum was given intranasally 2 days after 10^2 M.S.50 doses of virus. The serum had a hemagglutination-inhibition titer of 1:1024 against 8 units of PVM and completely prevented infection when given with or before the virus.

Effect of Immune Serum on the Course of PVM Pneumonia.—The capacity of Fr.B to inhibit multiplication of PVM and to modify the course of pneumonia induced with the agent has been postulated (2, 4) to be due to an effect of the polysaccharide upon the susceptible host cell. The polysaccharide, unlike specific antibody against the virus, does not combine with the agent and also does not prevent adsorption to susceptible cells (1, 2). If the mechanism of

action of Fr.B is as different from that of neutralizing antibody as has been thought, the injection of potent immune serum well after initiation of infection should not be followed by results similar to those obtained with the polysaccharide.

Experiments were carried out in a manner identical with that described in the preceding section. 0.05 cc. of PVM immune mouse serum, diluted 1:2 with saline and inactivated at 56°C. for 30 minutes, was injected intranasally in mice 2 days after the inoculation of 10^3 M.S.50 doses of PVM.

The results of a typical experiment are presented in Fig. 5. It is evident that there was no significant difference in either the concentration of PVM in the lungs of mice or the lesions induced by the virus whether immune serum was given at 2 days or was not given. The immune serum employed had a titer of hemagglutination-inhibiting antibodies of 1:1024 which correlates closely with the titer of neutralizing antibodies (8), and when injected a few hours before inoculation of the virus completely prevented infection of the mouse lung. It appears clear that, when given 2 days after inoculation of PVM, potent and specific immune serum had no demonstrable effect upon multiplication of the virus or upon the course of the pneumonic process and failed to reproduce the effects caused by Fr.B under identical conditions.

DISCUSSION

The evidence obtained in this study indicates that inhibition of the multiplication of PVM by the capsular polysaccharide of Friedländer bacillus is associated with a reduction in the extent of pneumonia induced with the virus. If viral multiplication is restricted by treatment at 3 days with the polysaccharide so that the concentration of the agent in the lung does not exceed about 10 per cent of the maximal value, the amount of pneumonia which develops does not go much beyond 55 per cent of the maximal value and most mice recover. If viral concentration is held, by similar treatment at 2 days, to about 3 per cent of the limiting value, then the extent of the lung lesion does not exceed 40 per cent of the limiting value and all mice recover. The data suggest that the extent of the pneumonic process is approximately what would be predicted (6) from the viral concentration attained and provide support for the hypothesis that active multiplication of PVM leads to abnormalities in the host cells which, in turn, result in the development of gross lesions. If the amount of pneumonia is a function of the degree of viral multiplication, a concept developed in the accompanying paper (6), it would be expected that a substance capable of inhibiting multiplication should also restrict the pneumonic process. It appears that this is the case.

It is noteworthy that specific neutralizing antibody injected 2 days after inoculation with the virus appears to have no effect upon multiplication or

upon the course of pneumonia induced with PVM. This finding lends further credence to the hypothesis that the polysaccharide does not act upon the viral particle, as does antibody, but instead exerts its effect by preventing the formation of new viral particles by the susceptible host cell.

That the polysaccharide inhibits some relatively late step in the formation of PVM is suggested by the finding that Fr.B inhibits multiplication of virus when injected as late as 10 hours after the beginning of the latent period of the multiplication cycle. It appears that the substance is effective when given at any time during the first two-thirds of the latent period but is ineffective after four-fifths of the period has been completed. The essential step, presumably blocked by the polysaccharide, is passed through by the multiplying virus before the 12th hour of the latent period but not before the 10th hour. Inhibition caused by the substance in the second or in subsequent cycles of multiplication would then be explained on a similar basis.

In studies on the effect of proflavine on the multiplication of bacterial viruses, T_2 and T_6, Foster (11) showed that inhibition was obtained when the substance was added during the first half of the latent period but not later in the cycle. Moreover, Cohen and Anderson (12) found that 5-methyltryptophane inhibited multiplication of T_2 virus only if the substance was introduced within 12 to 14 minutes after infection, which amounts to about half of the latent period. Studies now in progress in this laboratory indicate that a similar time relationship holds with mumps virus in the chick embryo; multiplication appears to be inhibitable by Friedländer polysaccharide during the first half of the latent period but not later in the cycle.

SUMMARY

Inhibition of the multiplication of PVM by the capsular polysaccharide of Friedländer bacillus, type B, is associated with restriction in the development of pneumonia induced with the virus in the mouse lung. The extent of the pneumonic process appears to be a function of the degree of viral multiplication; the greater the inhibition of multiplication, the less extensive is the pneumonia and the more probable is the recovery of animals treated with the polysaccharide. Effective therapy of pneumonia induced in mice with PVM is obtained with a single injection of 0.02 mg. of the substance given intranasally either 2 or 3 days after inoculation. Under appropriate conditions, treated animals recover completely from a viral infection which is, in control animals, uniformly fatal.

The polysaccharide produces inhibition if given in the first two-thirds of the latent period of the multiplication cycle, *i.e.*, within 10 hours, but is ineffective when given at 12 hours or later. However, the second cycle and subsequent cycles are inhibited irrespective of the time the substance is injected during the

first cycle of multiplication. The findings are discussed in relation to a theory regarding the mechanism of action of the polysaccharide.

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