EARLY PULMONARY LESIONS IN PARTIALLY IMMUNE ALCOHOLIZED MICE FOLLOWING INHALATION OF VIRULENT PNEUMOCOCCI

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In preceding papers (1) it has been shown that virulent pneumococci generally disappear from the lungs of normal mice within a few hours after inhalation and do not give rise to generalized infection. In alcohol-intoxicated mice, however, pneumococci persist in the lungs for a longer period and a fatal septicemia frequently follows. It has further been shown (2) that a high degree of immunity is produced in mice following repeated inhalations of live pneumococci and that a less marked immunity results from exposure to a spray of dead organisms. In the instances in which non-immune mice succumb as the result of infection following a pneumococcus spraying while alcoholized, death is due to general septicemia and there is no evidence of localized infection in the lung. However, mice which have been rendered partially immune by inhalation of live or killed pneumococci and are later alcoholized and exposed to virulent pneumococci show definite evidence of localized pulmonary infection.

In the foregoing experiments only animals which had succumbed to the pneumococcus infection were studied. Furthermore, the entire lung was not examined. There was the possibility that:—(1) some mice might have developed pneumonia but subsequently recovered, and (2) that early pneumonic lesions might have been missed as all the lobes were not examined histologically. In order to accurately determine the incidence of pulmonary localisation and to detect the initial pulmonary reactions in partially immunized mice following inhalation of virulent pneumococci while alcoholized, the following experiments were undertaken. This paper is a report on the examination of serial sections of the lungs of 81 partially immunised mice which

were allowed to inspire virulent homologous pneumococci while alcoholized.

Method

The mice to be immunized were placed in the spray chamber previously described and sprayed at intervals of 2 to 3 days with broth cultures of pneumococci which had been killed by heating to 60° for 30 minutes, or heat killed pneumococci suspended in salt solution.

Ten days after the last spraying the mice were alcoholized by intraperitoneal injection of 1.5 c.c. of a 10% solution of alcohol in saline. 1 hour after the administration of the alcohol, the animals were sprayed with 50 c.c. of virulent live pneumococcus culture and allowed to remain in the spray box for 1 hour. At intervals of 6, 12, 18 and 24 hours following spraying, groups of at least 9 mice were killed by chloroform.

After the heart's blood was cultured in broth, the lungs were removed in toto and dropped into Zenker's fluid to which 5% acetic acid had been added. They were paraffin embedded and serial sections were cut 12 microns thick. Every 12th section was examined as routine, and where lesions were discovered the intervening sections were examined.

The pulmonary lesion called chronic consolidation occurs in about 3 per cent of apparently healthy stock mice. Both in the gross and microscopically the lesions are readily differentiated from lesions due to the pneumococcus.

EXPERIMENTAL

Group I. 44 mice which had been partially immunised by spraying with whole killed cultures of pneumococci were exposed to inhalations of virulent type I pneumococci while alcoholized. The incidence of pulmonary lesions and the results of the heart's blood culture of these mice are given in Table I.

From this table it is seen that of the 10 mice killed in 6 hours, the lungs were normal in 4, and congested or edematous in 5. In only one animal was there any evidence of localisation of the infection as indicated by an inflammation in the alveolar wall.

The lungs of 7 of the 10 mice sacrificed in 12 hours appeared normal; in one chronic consolidation was present, in one congestion and edema, and in one the alveolar walls were inflamed.

The lungs of 6 of the 10 mice killed in 18 hours were normal; in one chronic consolidation and in three congestion were present.

The lungs of 10 of the 14 mice killed in 24 hours were normal; in 2 chronic consolidation occurred and in 2 congestion and edema.

In none of the mice killed in 6 hours was the heart's blood culture positive and the pneumococcus was recovered from only one and two mice of those killed in 12 and 18 hours respectively. 7 or 50% of the mice killed at 24 hours, however, showed positive heart's blood cultures.

Group II. 37 mice previously partially immunised by exposure to heat killed pneumococci were intoxicated and sprayed with virulent homologous pneumococcus. The results of the blood cultures and histological examination of the lungs of these mice are given in Table II.

TABLE I

Pulmonary Lesions in Lungs of Mice Partially Immunised by Inhalations of Heat-Killed Whole Cultures of Pneumococcus Type I

Time killed after inhalation of virulent Type I pneumococcus while alcoholised

6 hours			12 hours			18 hours			24 hours		
No.	Blood culture	Pathology	No.	Blood culture	Pathology	No.	Blood culture	Pathology	No.	Blood culture	Pathology
A1	−	C	B1	−	N	C1	−	N	D1	+	C.C.
A2	−	C.E.	B2	−	Early I.I.	C2	+	C	D2	+	C.C.
A3	−	H.E., C	B3	−	C.C.	C3	−	N	D3	−	N
A4	−	Early I.I.	B4	+	C., H.E.	C4	−	N	D4	−	N
A5	−	N	B5	−	N	C5	−	N	D5	+	N
A6	−	N	B6	−	N	C6	−	N	D6	−	N
A7	−	C	B7	−	N	C7	−	C	D7	−	N
A8	−	N	B8	−	N	C8	−	C	D8	−	N
A9	−	C.E.	B9	−	N	C9	+	C.C.	D9	−	N
A10	−	N	B10	−	N	C10	−	N	D10	+	N
									D11	+	C
									D12	+	C.E.
									D13	−	N
									D14	+	N

N = normal.
C = congestion.
E = edema.
H.E. = Hemorrhagic edema.
I.I. = Interstitial inflammation i.e. lesion limited to alveolar wall.
R = Red hepatisation.
C.C. = Chronic Consolidation.
− = negative blood culture.
+ = positive blood culture.

The lungs of 6 of the 9 mice killed in 6 hours appeared normal; in 2 they were congested and edematous and in one animal there was evidence of an attempt at localisation in the form of an inflammation in the alveolar wall.

The lungs of 8 of the 9 mice chloroformed at 12 hours were normal; in one they were congested and edematous and in one chronic consolidation was present. Localised areas of red hepatisation were found in 2 of the remaining 3 animals and interstitial inflammation was present in one.

Normal lungs were found in 5 of the 9 mice sacrificed at the end of 24 hours. Chronic consolidation occurred in 2 mice, congestion and edema in one, and red hepatisation in one.

The cultures of the heart's blood of all the mice killed after 6 and 12 hours remained sterile. Positive heart's blood cultures were obtained from 2 of the animals killed after 18 hours, while the blood of 5 or 55% of the mice sacrificed after 24 hours contained pneumococci.

TABLE II

Pulmonary Lesions in Lungs of Mice Partially Immunised by Inhalations of Heat-Killed Pneumococcus Type I Vaccine

Time killed after inhalation of virulent type I pneumococcus while intoxicated											
6 hours			12 hours			18 hours			24 hours		
No.	Blood culture	Pathology	No.	Blood culture	Pathology	No.	Blood culture	Pathology	No.	Blood culture	Pathology
A1	−	N	B1	−	N	C1	−	N	D1	−	C.C.
A2	−	N	B2	−	C.C.	C2	−	N	D2	−	N
A3	−	N	B3	−	N	C3	+	I.I.	D3	−	N
A4	−	N	B4	−	N	C4	−	Local R	D4	−	C.E.
A5	−	I.I.	B5	−	N	C5	−	N	D5	+	R
A6	−	N	B6	−	N	C6	−	C.C.	D6	+	N
A7	−	C.E.	B7	−	N	C7	−	N	D7	+	N
A8	−	N	B8	−	N	C8	−	N	D8	+	C.C.
A9	−	C.E.	B9	−	N	C9	+	Local R	D9	+	N
						C10	−	C.E.			

N = normal. I.I. = interstitial inflammation i.e. lesion limited to the alveolar wall.
C = congestion.
E = edema. R = red hepatisation.
− = negative blood culture.
+ = positive blood culture.

On gross examination the lungs of these 81 mice showed, in a few instances, congestion and chronic consolidation. Of these, 8 must be excluded because of the presence of chronic consolidation which would mask any slight lesions that might have been present. The lungs of 51 or 71% of the remaining mice were normal, and congestion and edema occurred in 15 or 21%. Evidence of pulmonary localisation occurred in 7 or 9%. The inflammatory process was limited to the alveolar walls in 2 mice killed after 6 hours, and in 2 animals killed after 18 and 24 hours respectively. In 3 mice exudation into the alveoli was the predominant feature and the lesions were classed as red hepatisation. Small areas of beginning con-

solidation occurred in 2 mice killed after 18 hours and the greater portion of one lobe was involved in a mouse killed in 24 hours.

Of the 81 mice, the pneumococcus was recovered from the heart's blood in 17 or 22%. Only one of the mice killed within 12 hours had a positive blood (5%), while 4 of those killed after 18 hours (20%) and 12 of the animals sacrificed after 24 hours showed a septicemia (52%). Of the 17 positive blood cultures, 4 occurred in mice with chronic consolidation and therefore must be excluded. Of the remaining 13, 6 occurred in mice with normal lungs, 4 were associated with congestion and edema, and 3 with inflammatory lesions.

Microscopic Pathology of the Early Pneumonic Lesions

This study is limited to serial sections of the 6 lungs mentioned above which showed inflammatory lesions not of the spontaneous chronic type often found in mice.

The nature of the early lesion is exemplified by the reaction occurring in the lungs of a mouse killed 6 hours after spraying:

An exudative inflammatory process involves 2 lobes, the caudal and ventral. The lesion is 2–4 m.m. wide, and does not reach the hilum nor the pleura. The inflammation is limited to the alveolar wall. The only exudation into the lumen has occurred at the junction of atria and bronchioli. Neither the large, nor small vessels nor the pleura are involved. In sections of this thickness (12 m.m.) it is not possible to say whether the thickening of the alveolar wall, with the accompanying exudate which is particularly rich in polymorphonuclear leucocytes, is due to inflammatory cells within or without the capillaries or to a combination of both. An area of atelectasis occupying about one fifth of the whole lobe is situated around the inflammation but the surrounding pulmonary tissue is congested. The area of inflammation appears to be continuous. The bronchial lymph nodes are not involved and no lymphatic involvement is apparent.

After 18 hours 2 lungs showed exudation into the alveolar lumen sufficiently marked to be considered the predominant lesion and were classed as stages of beginning red hepatisation.

DISCUSSION

The earlier studies on the production in the lower animals of lobar pneumonia with the pneumococcus were concerned at first with establishing the etiological rôle of this micro-organism.

The methods employed were direct injection of lung exudates into the thoracic cavity, trachea, peritoneum, and blood.

To Thalamon (3) is given the credit for first producing pulmonary lesions in rabbits by the intrathoracic method. Gameleia (4) carefully worked out the

relative resistance or susceptibility of the various lower animals to intraperitoneal injections of pneumococcus. He found that white mice and rabbits are most susceptible to pneumococcus infection while guinea pigs, dogs, sheep and man are increasingly more resistant. He produced pneumonia by intrathoracic inoculation in rabbits, dogs, and sheep. Gamaleia further showed that, since virulent strains would rapidly kill susceptible animals with an overwhelming septicemia without any pulmonary localisation, in order to produce pulmonary lesion in these animals an attenuated strain must be used. Because of this fact later investigators have attempted to determine what factors determine pulmonary localisation of the infection in susceptible animals. Wadsworth (5) expressed the belief that there exists a subtle equilibrium between the resistance of the animal on the one hand and the virulence of the organism on the other. He found that normal rabbits died of a fulminating septicemia without pulmonary lesions following intra-tracheal injections of highly virulent pneumococci. But on the other hand he could cause pneumonia to develop in normal animals by the use of less virulent cultures or by the use of virulent cultures in animals which had acquired a certain degree of immunity. In dogs, less susceptible animals, which were used by Lamar and Meltzer (6) and Wollstein and Meltzer (7), pneumonia was constantly induced after intra-bronchial insufflation of large amounts of culture. More recent work by Blake and Cecil (8) on monkeys has emphasised the bronchiogenic origin of the disease and these observers state that "lobar-pneumonia has been consistently produced in normal monkeys by the intra-tracheal injection of minute amounts of pneumococcus culture." Winternitz and Herschfelder (9), however, working on rabbits had previously found that it was necessary to introduce the organisms deep into the lower respiratory tract to produce pneumonia and Winternitz (10), considers that the lymphatic origin of the disease cannot be eliminated, in spite of the experiments quoted above, on the ground that by the method of intra-tracheal inoculation it is impossible not to infect the peribronchial tissue as the needle is being withdrawn.

It will be readily noted that all the "successful results" reported, without discriminating between those of doubtful and actual value, have been by intra-thoracic or intra-tracheal inoculation, there being no experimental proof of the haematogenous origin of the disease. But even the success of these methods has not been universally accepted, as many observers (Welch & Canfield (11), Krause and Pansini (12)) failed to confirm Gameleia's results, and succeeded only in producing lesions which they did not consider comparable to the typical lobar pneumonia as seen in man.

The present method, that of inhalation, is generally admitted to have failed, although some investigators believed that an occasional positive result was obtained. Selter (13) was unable to produce pneu-

monia in rabbits and guinea pigs exposed in a box to a spray of pneumococci which had been isolated from an epidemic of pneumonia in these animals. The inhalation method was employed in the present experiments because it is less artificial than the others and removes the objection of mechanical injury.

The points which must be considered in any discussion of the pathogenesis of pneumonia are: 1—the route by which the causative agent reaches the lung and 2—the site of initial invasion and spread of the infection in the pulmonary tissue.

Mode of Invasion

The infecting organism may reach the lungs by the blood stream, lymphatics, or directly from the respiratory tract.

The lymphatic route presupposes that entrance takes place in the bronchi or upper respiratory tract and that the organisms progress along the lymphatics to the lymph nodes at the root of the lung. The lymphatics become blocked by the inflammatory reaction set up by the organisms causing a back pressure and the infection is first noticed in the lung tissue. Or the organisms may pass through the lymph nodes into the thoracic duct and reach the lung by the pulmonary artery. In the mouse the lung is scant in lymphatics and we have seen no evidence in our sections of any such marked lymphatic involvement as this mode of infection would presuppose. It is certain that the lymph nodes themselves are not early affected.

The occurrence of pulmonary changes in the presence of negative blood cultures in the first 6 hours would at first sight appear to favor theory of direct primary invasion of the pulmonary tissue itself, particularly as the earliest lesions encountered have always been in the alveolar wall. But these pathological findings may be equally well explained on the ground that the infecting agent reaches the alveolar wall by the capillaries and is localised there until later when a secondary septicemia occurs.

The adherents of the hematogenous method of infection assume that the pneumococcus gains entrance at some point in the respiratory tract and is carried either directly into or through the lymphatics into the blood stream to the lungs where the microorganisms set up a

pneumonia. A selective filtration action on the part of the lungs is assumed to take place. No direct experimental evidence has yet been brought forward to prove this hypothesis. Attempts to test its validity are now in progress. The fact that pneumococci may be recovered from the liver, spleen and kidney of rabbits and guinea pigs following inhalation of pneumococci, though the blood culture is negative, indicates that a transient pneumococcus septicemia may occur. It has further been shown by daily blood cultures following spraying that a transient pneumococcus septicemia occurs frequently in rabbits. There probably are few organisms free at any one time in the circulating blood and furthermore these few bacteria may be within leucocytes, not multiplying in the blood. The occasional organisms which reach the blood are probably rapidly filtered out by the tissues and locally destroyed.

The failure to obtain positive blood cultures in the 2 mice which showed evidence of pulmonary lesions may be due to the circumstance that re-invasion of the blood stream from these foci of infection had not taken place after, possibly, transient primary septicemia. 2 of the 4 mice killed after 18 hours, however, with lungs showing lesions, had a generalised septicemia.

Nature of the Initial Lesion

In a proportion of partially immunised and intoxicated mice, infection after inhalation of virulent pneumococci is localised in the lungs. In these mice the initial lesion is an inflammation of the alveolar wall (extra or intra vascular). Blake and Cecil incline to the belief that the early lesion is in the interstitial tissue, while Permar (14) speaks of the disease as an exudative alveolitis throughout. Since cellular exudation into the alveoli occurs very early, the nature of the initial lesion can only be determined in mice killed soon after infection. But these early lesions are so minute that there is no gross pathological change except perhaps congestion, the significance of which is debatable in sacrificed animals. The only safe method is to cut serial sections of the whole lung in order to avoid missing any minute early lesions.

In the present study localisation of the pneumococcus infection was present in the lungs of 7 of 71 mice. Although this is too small a

number of animals from which to draw definite conclusions, the outcome agrees with that of previous experiments in which the observations were made on mice which had succumbed to the infection. The lymphatic spread from the original focus as observed in monkeys by Blake & Cecil certainly does not occur in the mouse. The infection in this species is progressive and is localized to the alveolar wall. In previous experiments pleurisy occurred when extensive lymphatic involvement was found and the animals died before a well defined localisation had time to develop. We believe that experimental pneumococcus pneumonia, at least in the mouse, begins in the alveolar wall, possibly within the capillaries.

SUMMARY

1. The serial section examination of 71 partially immune alcoholised mice which were killed at intervals following the inhalation of virulent pneumococci showed pulmonary localisation in 7 or 9%.

2. In the case of the mouse the initial lesion of pneumococcus pneumonia is in the alveolar wall and the exudate into the alveolar lumen occurs secondarily.

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