REACTIONS OF RABBITS TO INTRACUTANEOUS INJECTIONS OF PNEUMOCOCCI AND THEIR PRODUCTS

VII. THE RELATION OF HYPERSENSITIVENESS TO LESIONS IN THE LUNGS OF RABBITS INFECTED WITH PNEUMOCOCCI

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Certain writers have drawn attention to the effect of active immunization or sensitization (1–6) on the occurrence or character of lesions in the lungs of animals artificially infected with pneumococci. On the basis of these observations some investigators have attempted to explain the pathogenesis of pneumonia in man. In a recent paper, Stuppy, Cannon, and Falk (7) report that after daily insufflation of heat-killed pneumococci, rabbits acquire a type-specific immunity and the reaction of the lung following intratracheal injections of cultures of corresponding types consists of proliferation and exudation with the macrophage the predominant cell. Polymorphonuclear and eosinophilic cells were also present. A similar though less extensive reaction was induced by the intratracheal injection of cultures of heterologous types. Sharp and Blake (8) have shown that the intratracheal injection of an autolysate of Pneumococcus induces no reaction in the lung of normal rabbits but in rabbits previously inoculated with bacteria or autolysate similar injections stimulate inflammatory changes in the lung which are parallel in severity with the skin reactivity of these rabbits to the same material.

In the previous papers of this series, studies have been reported which indicate that rabbits undergo different forms of sensitization to pneumococci and their constituents depending upon the route and mode of injection. It has been shown that when rabbits receive repeated intracutaneous or intravenous injections of pneumococci or solutions of their protein constituents (9), they acquire a heightened sensitivity, so that when later intracutaneous injections of nucleoprotein are made, a local reaction occurs which is similar to that seen in the Arthus phenomenon. This skin sensitivity does not seem to be associated with a state of increased resistance to infection.

In rabbits that have been repeatedly injected intracutaneously with suspensions of dead pneumococci, there occurs in addition to the heightened skin reactivity, a condition of heightened eye sensitivity (10) which is manifested by an inflammatory reaction when there is instilled in the eyes a solution of pneumococcus nucleoprotein or a solution of pneumococci from which the acid- and heat-coagulable proteins have been removed. It was found that the eye sensitivity does not occur.
when the previous injections are made by routes other than the skin, and it develops only after intact bacteria are injected. The heightened eye reactivity apparently occurs only in animals that are resistant to infection.

In the light of these observations, it has seemed important to investigate further the possibility of sensitization of the tissues of the lung to pneumococci and their products and to study the relationship of this phenomenon to actual infection with pneumococci.

The first experiments bearing on the problem consisted in determining whether rabbits which had been sensitized previously by repeated injections of a foreign protein would show an increased reaction in the lung following the intratracheal injection of the same protein.

Opie (11) has called attention to the occurrence of hypersensitivity in tissues other than the skin, and has described the occurrence of localized consolidation in the lung following the injection of 0.2 cc. of horse serum through the thoracic wall in a rabbit which had previously received repeated injections of serum. Accordingly, rabbits were injected repeatedly either intracutaneously or intravenously with crystalline egg albumin as previously described. Three weeks after the last injection the skin reacted severely to injection of the homologous protein but no sensitivity of the eye could be demonstrated. After observations had been made on eye and skin reactivity, solutions of egg albumin containing 3 to 10 mg. of the protein were injected intratracheally and the animals were killed 24 hours later by the intravenous injection of air. Upon histological examination, the lungs showed different degrees of reaction. In some instances definite pneumonic areas were observed. The alveolar walls were infiltrated and the alveoli were filled with an exudate containing chiefly mononuclear and polymorphonuclear cells. In other instances no definite changes were seen.

It is obvious from this experiment that certain rabbits which have received repeated injections of a foreign protein are so sensitized that an acute reaction occurs in the lung when the protein solution is reinjected intratracheally.

It was now important to repeat this experiment, employing however, instead of egg albumin, the nucleoprotein derived from pneumococci. Using the same technique, similar results were obtained.

Another series of animals were now sensitized by repeated intravenous or intracutaneous injections of whole heat-killed pneumococci, instead of merely the bacterial protein. On subsequent intratracheal injections of the bacterial protein, these animals reacted just as did those in the previous experiments.
Experiments were then undertaken to determine whether this state of hypersensitiveness, the presence of which could be demonstrated by the intratracheal injection of protein, could be passively transferred from one animal to another by the injection into a normal animal of the serum from a sensitized one.

Each of nine normal rabbits was injected intravenously with 14 to 30 cc. of serum derived from a single animal which gave typical skin reactions to the respective antigens. Three of the rabbits received serum from rabbits injected with egg albumin, three received serum from rabbits injected with pneumococcus protein, and three received serum from rabbits injected with heat-killed pneumococci. On the day following the transfer of serum, the recipient animals were found to possess precipitins in their sera and exhibited reactions in the skin following injections of the specific antigen. Eye sensitivity was tested but in no instance was a positive reaction obtained. The introduction of the respective protein intratracheally induced in the lung a lesion comparable to that observed in the actively sensitized animals. In three of the animals the reaction in the lung was of the more severe variety while in the others the reaction was mild and diffuse or even doubtful.

An attempt was made to determine whether the lung reaction could also be elicited by the intratracheal injection of a solution of pneumococci from which the acid- and heat-coagulable proteins had been removed. The reactions produced in normal rabbits by this material were so severe, however, that no conclusions could be drawn.

These experiments indicate that in rabbits a state of sensitization to foreign proteins, including pneumococcus nucleoprotein, may be induced so that upon the intratracheal injection of this protein inflammatory reactions occur in the lung parenchyma. That this sensitivity may be passively transferred, and that it follows both intravenous and intracutaneous inoculations indicate that it is analogous to the heightened skin reactivity in rabbits (12), and that it differs from the eye sensitivity previously described.

Experiments were then undertaken to determine, if possible, the effect of such a state of hypersensitivity on actual infection with pneumococci.

Preliminary studies in normal animals, employing the same technique as was used later in the sensitized ones, showed that following the intravenous injection of small quantities (10^-6 to 10^-7 cc.) of broth cultures of Pneumococcus, the animals
died usually within 24 to 48 hours, more rarely 72 hours, with a marked septice-
mia. The lungs showed varying degrees of involvement. Frequently there were
found inflammatory reactions, alveolar exudation, and in some instances, hemor-
rhages. Rabbits which had previously received repeated intravenous or intracu-
taneous injections of heat-killed pneumococci were not similarly infected. As
these animals were more or less resistant to the infection they were killed in from
1 to 5 days following inoculation by the intravenous injection of air. The majority
of these animals showed little or no change in the lung. In those dying 4 to 5 days
following the infection, however, quite marked changes were frequently observed.
In the lungs of some of these rabbits there was a distinct pneumonic process con-
sisting of an enormous increase of mononuclear and polymorphonuclear leucocytes.
These cells filled the alveoli distending the walls to form an area of consolidation.
Occasionally bronchioles were found containing numbers of necrotic cells. Usu-
ally, the cellular reaction was principally mononuclear. In a small number of
animals dying of the infection, extensive empyema and massive pericarditis was
found.

Even in these partially resistant animals the lesions differed from
those seen in previously untreated rabbits merely in severity and in the
occasional occurrence of empyema and pericarditis.

As previously stated, only certain of the animals showed lesions in
the lungs. It was established that it could not be predicted which of
the animals would show pulmonary lesions and which would not.
Certain of the animals were prepared by repeated intracutaneous
injections of whole pneumococci, and in the majority of instances, these were eye sensitive. There was no difference in the character or
frequency of the pulmonary lesions in the animals which were eye
sensitive and in those which were not.

Finally an attempt was made to determine whether when infection
was induced in rabbits already undergoing a pulmonary protein re-
action, the lesions in the lung would be markedly different from those
occurring in the similarly infected normal animal. For this purpose
rabbits were previously injected either intracutaneously or intrave-
nously with heat-killed pneumococci until their sera showed a high
titre of antiprotein precipitins. About 3 weeks after the last injection
of bacteria, pneumococcus protein was introduced intratracheally and
24 hours later virulent cultures of the organism were injected intra-
venously. A study of the lung reaction to infection in these rabbits
showed no essential difference from that occurring in sensitized rab-
bits which had not received nucleoprotein intratracheally on the
preceding day.
DISCUSSION

The studies reported in the present communication were undertaken to determine whether a preexisting state of hypersensitiveness to Pneumococcus or its products influences the course and character of pneumococcus infection in the lungs of rabbits. The study was preceded by observations on the reaction of the lung to soluble proteins in sensitive animals. It was found that the intratracheal injection of native or pneumococcus proteins may induce inflammatory reactions of the lung in animals previously injected with the respective antigen. Moreover, similar reactions follow the intratracheal injection of pneumococcus protein in rabbits that have previously received inoculations of heat-killed pneumococci. That the reaction induced by intratracheal injections of protein is related to the presence of circulating precipitins was shown by the occurrence of the lung reaction, following the transfer of serum from a reactive to a normal rabbit.

A study of the response of the lung to infection in animals made resistant or sensitive by the repeated intravenous or intracutaneous injections of pneumococci shows no appreciable difference attributable to the route of preliminary administration of the bacteria. Infection was accomplished by the intravenous injection of many lethal doses of virulent organisms, and the reaction in the lung occurred neither frequently nor regularly. As far as could be determined by this method of infection the response of the lung is not different in normal animals and in rabbits which have previously received intravenous or intracutaneous injections of pneumococci. The presence of eye or skin hypersensitiveness appears to have no influence on the course or character of the induced infection.

In order to test the possibility that an antigen-antibody reaction in the lung might increase the severity of the infection, pneumococcus protein was first injected intratracheally in sensitized rabbits and on the following day the rabbits were infected by the intravenous injection of pneumococci. The presence of an intrapulmonary reaction at the time of infection did not have any apparent effect on the course of the disease.

In brief, it can be stated that the present study fails to show that hypersensitiveness to Pneumococcus or its products influences the occurrence or character of the lesions in the lungs in artificial pneumococcus infections.
SUMMARY AND CONCLUSION

1. The intratracheal injection of egg albumin or pneumococcus protein induces an inflammatory reaction in the lungs of rabbits previously inoculated with the respective antigen.

2. A similar reaction occurs following intratracheal injection of pneumococcus protein into the lungs of rabbits previously inoculated with heat-killed suspensions of the bacteria.

3. This reaction appears to be related to the presence of circulating antibody and to have the nature of the Arthus reaction.

4. A study of the reaction of the lung of rabbits to infection caused by intravenous injections of Pneumococcus reveals that (a) reactions occur irregularly in the lung; (b) in the lungs in which reactions do occur, the histological changes are not different in normal rabbits and in rabbits made resistant by previous intravenous or intracutaneous injections of pneumococci.

5. Intratracheal injection of pneumococcus protein followed by intravenous injection of virulent pneumococci on the next day does not alter the course and character of the infection in resistant rabbits.

6. The experiments reported in this paper bring no evidences to support the view that the lesions in the lungs of rabbits following the intravenous injection of pneumococci are modified by any previous state of sensitivity.

REFERENCES