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Abstracts of "Tuberculosis Research"

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Experimental Studies on Desensitization in Tuberculosis.

I. Correlation between tuberculin allergy and circulating antibody

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In order to elucidate the mechanism of tuberculin allergy, a study was made on the effect of specific desensitization on the behavior of the tuberculin type skin reaction, on the three different kinds of circulating antibodies and on the specific cytolysis of splenic cells.

Rabbits sensitized with heat-killed bacilli were desensitized with Old Tuberculin (OT), purified protein derivative (PPD-s), some kinds of somatic protein fractions and heat-killed tubercle bacilli, respectively.

The following conclusions were obtained.

- 1) Of the three different kinds of antibodies, antipolysaccharide, antiphosphatide, and antiprotein, only the antiprotein significantly increased in the course of the desensitizing procedure.
- 2) Animals once desensitized to OT remained sensitive to PPD-s and the protein fractions.
- 3) No specific cytolysis was observed in animals repeatedly injected with anyone of the antigens used in this experiment.
- 4) No direct relationship was found to exist among the amount of anyone of the three antibodies, the specific cytolysis of splenic cells and the tuberculin type skin reaction.

II. Comparison of the tuberculin type skin reaction in guinea pigs and rabbits.

In this study comparison was made between rabbits and guineapigs in connection with the tuberculin type skin reaction, the specific cytolysis, and the circulating antibody, when they were sensitized with dead tubercle bacilli and then subjected to the same desensitizing treatment.

For the purpose of desensitizing the animals, use was made of OT, PPD-s and somatic protein fraction of tubercle bacilli, BR-4b.

The following findings were obtained.

- 1) Similar to the results obtained in the preceding study, no correlation was noticed in the rabbits between the amount of circulating antibodies and the degree of the tuberculin type skin reaction.
- 2) In case of the desensitizing treatment using PPD-s, differences in skin reactivity were noticed between guineapigs and rabbits; in the former the skin reactivity disappeared or diminished only to PPD-s, while in the latter only to OT.
- 3) Neither in guineapigs, no correlation was found to exist between the specific cytolysis of splenic cells and the tuberculin type skin reaction.

III. Comparison of the protein fractions isolated from culture filtrates of tubercle bacilli with those from tubercle bacillus cells.

In the preceding studies, it was suggested that there might exist certain differences in the desensitizing effect between protein fractions isolated from culture filtrates and somatic protein fractions.

In this experiment, the effect of two different protein fractions, somatic and of culture filtrate on the circulating antibody, the specific cytolysis of splenic cells, and the tuberculin type skin reaction was studied. Rabbits sensitized with either PPD-s or a protein fraction isolated from tubercle bacilli (Bpr) respectively, were crossly treated by the desensitizing procedure using PPD-s and Bpr.

The following results were obtained.

- 1) In all the groups of the animals treated, disappearance or diminution of the skin sensitivity to OT was noticed. In contrast, in the animals sensitized with anyone of the two protein fractions, and then repeatedly treated with the same fraction, the skin reactivity remained unchanged to the same fraction, while in the animals repeatedly treated with the other heterologous fraction, increase in the skin reactivity to this heterologous fraction was remarkable.
- 2) In all the groups of the animals, no correlation was noticed among the circulating antibody, the specific cytolysis of splenic cells and the tuberculin type skin reaction.

Experimental Study on the Antigenical Diversity of the Tuberculoprotein

III. Comparison of Tuberculoproteins Obtained by Various Precipitating Procedures.

Harue OKUYAMA, Akihiko OHTA and Kazuo MORIKAWA

Different samples of tuberculoprotein were precipitated from non-heated Sauton culture filtrates with various precipitating agents, such as trichloroacetic acid, acetic acid and ammonium sulfate. The serological and biological activities of these proteins were compared with each other, and the following results were obtained.

The protein yield was largest when trichloroacetic acid was used, especially at room temperature.

The precipitating antigenicity of the ammonium sulfate-precipitated protein against tuberculous rabbits was the highest, that of the acetic acid-precipitated protein the lowest.

The activity of these proteins to elicit tuberculin skin reaction in tuberculous rabbits was approximately of the same degree when they were injected at doses of equal nitrogen concentration.

By the use of these proteins the tuberculin reaction was evoked in rabbits sensitized with either tuberculoprotein, or acetone-killed tubercle bacilli or heat-killed tubercle bacilli. From these experiments the tuberculoprotein was found to be different in sensitizing antigenicity from other antigens: the tuberculoprotein induced the immediate type of skin reactivity, while heat-killed bacilli the delayed type and acetone-killed bacilli the intermediate type.

An Experimental Study on the Adjuvant Effect on Ovalbumin Sensitization of the Proteolipid Fraction from *Myc. tuberculosis*

Eiji HAMADA and Kaoru KAWACHI

In order to examine the adjuvant effect on ovalbumin sensitization of the proteolipid fraction (Folch) from *Myc. tuberculosis*, rabbits were injected subcutaneously with ovalbumin alone or together with the proteolipid fraction or Freund's complete adjuvant containing killed tubercle bacilli. At given intervals after sensitization the skin sensitivity and the serum antibody against both ovalbumin and tuberculoprotein were measured. The results obtained were as follows :

1. When the proteolipid fraction mixed with ovalbumin was used as the sensitizing antigen, the skin sensitivity provoked against ovalbumin was almost of the same degree as in the case of sensitization with ovalbumin alone. However, the precipitating antibody titer was considerably higher.
2. When ovalbumin was injected together with complete adjuvant, the skin sensitivity as well as the serum antibody production were markedly intensified. In this group of animals transient skin hypersensitivity of the delayed type against ovalbumin was demonstrated until 9 days after sensitization, followed by gradual change into the immediate type.
3. In the rabbits injected with the complete adjuvant, a high degree of skin sensitivity of the delayed type as well as a high antibody titer were shown against tuberculoprotein. On the contrary, in the rabbits injected with the proteolipid fraction the skin sensitivity to tuberculoprotein was observable only from 30 days after sensitization, and the precipitating antibody against tuberculoprotein was not detectable until 37 days after sensitization. Moreover, skin sensitivity thus provoked was of the immediate type rather than of the delayed type.
4. From the above findings it is concluded that the proteolipid fraction has an adjuvant effect for production of precipitin against ovalbumin.

Immunopathological Studies on Aspergillosis

I. Antigen Preparation and Allergic Reaction Caused by Heat-killed

Aspergillus fumigatus

Kaoru KAWACHI

- I. Attempts were made to fractionate immunologically active materials from mycelia and culture filtrates of a pathogenic fungus, *Aspergillus fumigatus*.
- 1) A protein fraction was obtained from culture filtrates by precipitation with 80 per cent saturated ammonium sulfate. This fraction was found to be a good antigen for allergic skin test in rabbits sensitized with killed aspergilli as well as for precipitation reaction with anti-*Aspergillus* sera.
- 2) Using the passive cutaneous anaphylaxis technique rabbit anti-*Aspergillus* sera were found to contain skin sensitizing antibodies.
- II. In order to study the role of sensitization in the development of aspergillosis lesions, heat-killed aspergilli were injected intravenously both into rabbits previously sensitized with the same organisms and into normal

rabbits. After injection they were sacrificed at given intervals and their lung lesions were observed histologically and comparatively.

- 1) In the sensitized animals severe diffuse lesions developed in the early stage after injection, presenting intense bleeding and marked polymorphonuclear and mononuclear infiltration, followed by development of proliferative inflammatory lesions composed of epithelioid cells.

On the contrary, in control animals only slight lesions corresponding to the foreign body reaction took place in the early stage. These lesions were absorbed rapidly.

- 2) Serologically no correlation was found to exist between the precipitin titers and the degree of the lesions.
- 3) The above results are considered to suggest that sensitization plays an important role in the development of aspergillosis lesions.