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## Abstracts of "Tuberculosis Research"

Vol. 17/18, 1963

### Experimental Studies on Allergy in Tuberculosis by the Use of the Suspension Culture Technic

Ken-ichi YAMAMOTO, JUN ARIMA, Akio SASAKI and Yoshio TAKAHASHI

In order to elucidate the mechanism of tuberculin allergy, study was made, using the suspension culture technic, of the various antigenic factors involved in specific cytolysis of splenic cells.

The following conclusions were drawn.

- 1) Among antigenic fractions tested in this study, almost all protein fractions isolated from either cells of tubercle bacilli or culture filtrates were found to produce a specific cytolysis of immune splenic cells, whereas polysaccharide and phosphatide fraction in comparable dilutions had no specific injurious action, except for one of the tuberculin polysaccharide fractions, Ts-2.
- 2) Anti-allergic agents, acidomycin, glycyron and prednisolone, as well as complement-inactivating agents, EDTA and sodium citrate, inhibited the cytolytic action of the protein fractions upon splenic cells from guinea pigs immunized with heat-killed tubercle bacilli.
- 3) This cytolysis was also inhibited, when the splenic cells had been previously treated either in vitro or in vivo with polysaccharide or phosphatide fractions.

This inhibition to cytolysis of splenic cells sensitized with human tubercle bacilli was observed only following administration of polysaccharide or phosphatide fractions derived from human bacilli but not following administration of similar fractions isolated from *M. Phlei* or anonymous acid-fast bacilli.

### Experimental Study on the Antigenical Diversity of Tuberculoprotein

#### II. Digestion of the Tuberculoprotein Fractions with Proteolytic Enzyme

Harue OKUYAMA, Akihiko OHTA and Kazuo MORIKAWA

A tuberculoprotein was obtained from non-heated culture filtrates of tubercle bacilli by precipitation with trichloroacetic acid at pH 4.0, and fractionated chromatographically with DEAE-cellulose.

Each fraction gave a positive precipitation against a given antiserum, only when its undiluted eluate was used, except for one fraction which was positive at a ten-fold dilution. On the contrary, the precipitin titer of the antiserum differed from one fraction to another. This suggested that the protein fraction contained antigenically different substances. The possibility of these antigenic substances being of protein nature was considered to be scarce, because the precipitin titers were hardly affected, when they had previously been treated with trypsin. From the ability to produce skin reactions, the tuberculoproteins were separable into three groups: one which showed a maximum reaction at 6th hour after injection, i.e. an immediate type of reaction, the second which showed a mixed form of immediate and delayed reactions, and the third which showed a typical delayed type of reaction. The skin reaction to the first group was of polymorphonuclear type, while that to the third group was of mononuclear type. The skin reaction of delayed type was suppressed in intensity, when the corresponding was previously treated with trypsin.

From the above results, it is supposed that the tuberculoprotein participating in precipitation may differ from that eliciting the skin reaction.

## Allergic Reaction in the Lungs--An Immunopathological Study

### 3. Allergic Reaction against Coagulated Ovalbumin

Kaoru KAWACHI and Masako TOMISAKI

Normal rabbits were sensitized with ethanol-coagulated ovalbumin.

After sensitization they showed a high skin sensitivity as well as a high serum precipitin titer against the original ovalbumin.

Seventy days after sensitization these animals together with normal control rabbits were intratracheally reinjected with the same antigen.

After reinjection they were sacrificed at given intervals and their lungs were histologically studied.

In the sensitized animals severe exudative lesions were formed in the early stage, followed by a few tuberculoid lesions composed of large mononuclears. Ten days after reinjection these mononuclears began to form proliferating inflammatory lesions, the lesions being, however, slight in volume and in number.

On the contrary, the control animals showed only weak reactions corresponding to the foreign body reaction.

## Allergic Reaction in the Lungs--An Immunopathological Study

### 4. Reinjection of Killed Tubercle Bacilli

Kaoru KAWACHI and Masako TOMISAKI

Rabbits previously sensitized with heat-killed tubercle bacilli together with normal rabbits as control were intratracheally reinjected with the same material. They were sacrificed at given intervals for histological examinations of their lungs.

1. Following sensitization with killed bacilli all rabbits showed a high skin sensitivity to tubercle-protein. In these animals, high serum titers were observed for antiprotein and antiphosphatide antibodies.

2. Intratracheal administration of killed bacilli caused a marked decrease in the sensitivity.

3. In the sensitized animals a severe exudative inflammatory reaction was observed in the early stage following reinjection. Five days after reinjection immature epithelioid cells developed. From the 7th day tubercles were formed, and from the 10th day proliferative inflammation took place, showing a large quantity of mature epithelioid cells.

4. On the contrary in the non-sensitized animals, pulmonary changes were only slight, and a few tubercles constituting of immature epithelioid cells were formed on the 15th day after reinjection.

## A Comparison of the Reactions with Purified Tuberculin (PPD-s) and Old Tuberculin (OT)

Shigeichi KAWAMURA

In a certain city in Hokkaido, 371 school children were tuberculin tested with the dose of 0.05 $\gamma$  of PPD-s and of 0.1 ml of 1/2000 diluted OT and the reactions in the tuberculin non-tested and re-tested skin were observed.

- 1) Compared by the ratio-value, the titer of PPD-s seemed to be almost equivalent to that of OT.
- 2) According to the criteria for the reading of tuberculin reaction there was no difference between the rate of the positive reaction by PPD-s and that by OT, and also the frequency distribution curves of the size of erythema ran almost parallel.
- 3) The proportion of the recognizable induration was a little higher in PPD-s than in OT.
- 4) P-reaction, k-reaction and faint reaction, etc. in the tuberculin re-tested skin was significantly less in PPD-s than in OT.
- 5) In the tuberculin re-tested skin both of PPD-s and OT showed an accelerated reaction.

## Comparison of the Takahashi Phosphatide-Kaolin Agglutination Test with the Gel Double Diffusion Test in the Diagnosis of Human Tuberculosis

Ei-ichi SAKAI

The gel double diffusion test postulated by Parleet and Youmans was studied with 1455 samples of serum obtained from tuberculous, non-tuberculous patients and healthy person, using, as the antigens, samples of Sauton-tuberculin, polysaccharide and protein fractions isolated from Sauton-tuberculins and tubercle bacilli, and a phosphatide fraction obtained from tubercle bacilli.

When Sauton-tuberculins were used as the antigens, the diffusion test was found to be positive at 70 to 80 percent for active tuberculous patients and 15 to 25 percent positive for inactive patients.

When polysaccharide and protein fractions of tuberculin or bacillary origins were used separately as the antigens, no appreciable difference was noticed between tuberculous and non-tuberculous patients, the test having been positive at 70 to 100 percent in both groups.

When bacillary phosphatide was used as the antigen, the test was about 60 percent positive for active tuberculous patients, while it was only about 8 percent positive for healthy persons, thus indicating the preference of bacillary phosphatide as antigen for the diagnosis of activity of human tuberculosis.

The Takahashi phosphatide-kaolin test was proved to be most preferable for the diagnosis of human tuberculosis: its percentage in tuberculosis patients was higher than that of gel test and it reflected more faithfully the degree of tuberculous infection.

Most of the gel-positive person who had kaolin-titers higher than 16 were proved to have active pulmonary tuberculosis.