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## Abstracts of "Tuberculosis Research"

Vol. 25/26, 1967

### Studies on the Constituents of the Tubercle Bacillus with Special Reference to Their Role in the Development of Allergy and Immunity

Shuichi OHASHI

Immunological studies were made in the rabbit of protein, polysaccharide, phosphatide and wax fractions obtained from tuberculin and tubercle bacilli. They were injected intramuscularly into the animals, alone or combined, after being incorporated in water-oil adjuvant.

Several weeks after the injection of the antigens, the animals were challenged with live virulent tubercle bacilli. The animals were studied for several weeks of their sensitization to tuberculin and formation of circulating antibodies to the injected antigens. After that they were sacrificed and the visceral tuberculous lesions were compared with those formed in normal control animals.

Convincing evidence is given that tuberculin allergy and immunity in tuberculosis are two distinct phenomena of different mechanisms, the former being caused to take place by protein and the latter by polysaccharide. However, the possibility of the allergic state provoked by protein playing a certain role in the development of immunity caused by polysaccharide can not be excluded.

Both phosphatide and wax have nothing to do directly with the development of allergy and immunity. Wax is endowed, however, with an ability to enhance tuberculous lesions.

### Biochemical Studies on the Erythrocyte-Sensitizing Ability of the Phosphatide of the Tubercle Bacillus

Akio SASAKI and Yoshio TAKAHASHI

The antigenicity of the phosphatide of the tubercle bacillus was analysed by means of various biochemical fractionation techniques.

Acetone-killed BCG bacilli were first extracted with acetone in the cold, then the residual bacilli were subjected to extraction with methanol at room temperature. A sample of crude methanol extract thus obtained was fractionated into several subfractions, after having been removed of its water-soluble ingredients, and a phosphatide fraction was obtained which was able to sensitize sheep erythrocytes at as low a concentration as 1  $\mu$ g per ml of 2 percent erythrocyte suspension. It was confirmed that the strong erythrocyte-sensitizing ability of this phosphatide fraction was not due to the presence of a minute quantity of polysaccharide in the fraction. Silicic-acid column-chromatograms of phosphatide fractions obtained by extraction with mixtures of chloroform and methanol gave strong evidence that the erythrocyte-sensitizing ability consists in the inositol-phosphatidyl moiety of the fractions.

Antituberculous Compound. XXV.  
Some Derivatives of Pyrido [2,3-d] pyridazine  
Shichiro KAKIMOTO and Shuichi TONOOKA

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Attempts to prepare 5,6-dihydropyrido [2,3-d] pyridazin-8 (7H)-one by the hydrogenation of pyrido [2,3-d] pyridazin-8(7H)-one were not successful, but it was found that the hydrogenated compound was 1,2,3,4-tetrahydropyrido [2,3-d] pyridazin-8(7H)-one. The same reaction occurred in the case of pyrido [2,3-d] pyridazin-5(6H)-one and some other derivatives.

Immunological Studies on Aspergillosis  
IV. Influence of sensitization on the phagocytic activity

Kaoru KAWACHI

In order to determine the role of sensitization in the course of infection of aspergillosis, investigation were made of the effects of sensitization on the phagocytic and digestive activity of the host phagocytes on the basis of cellular and humoral factors.

Rabbits previously sensitized with killed aspergilli and normal rabbits were both challenged intraperitoneally with living aspergillus spores, and then the phagocytic activity of the intraperitoneal cells was compared at intervals between the two groups of animals. In parallel, the phagocytic activity of the intraperitoneal cells removed from sensitized and non-sensitized rabbits was observed in vitro in the presence of normal and immune rabbit serum.

Significant difference in phagocytic activity was observed between sensitized and normal rabbits both in vivo and in vitro, i. e., sensitization enhanced the phagocytic activity for aspergilli. However, addition of immune serum in vitro had little effect on the phagocytic activity. These findings agree with the result obtained in a previous study that pulmonary pathologic changes were less intense in sensitized animals.

Immunological Studies on Aspergillosis  
V. General discussion and conclusions

Kaoru KAWACHI

For the purpose of getting information of the influences of sensitization of host animals on infection and manifestations of aspergillosis, the following experiments were undertaken: purification of antigens for conducting immunological reactions, superinfection experiments in rabbits using live and dead aspergillus fungi, measurements of the phagocytic activity of sensitized cells and antibody analysis of rabbit immune sera as well as patients sera by the use of DEAE-cellulose chromatography. The study led to the results in the following:

Protein fractions extracted either from fungi or from their culture filtrates were proved to have the best antigenicity.

Previous sensitization exerted a great influence on the formation of pulmonary lesions by the fungi: rapid productive and proliferative inflammation come to the force, resulting in the hindrance of dissemination of the challenged fungi. The validity of the foregoing findings was supported by the increase in the ability of phagocytosis and digestion of the macrophages of the challenged animals. Furthermore, analyses of the circulating antibodies assumed the participation of skin sensitizing antibodies in the formation of fungal lesions.

### Study on Antibody Formation

## II. Development of $\gamma$ G and $\gamma$ M Cells in Antituberculin Antibody Response

K. MORIKAWA, H. OKUYAMA, K. KAWACHI,  
E. HAMADA & A. TAKAHASHI

In order to research the development of  $\gamma$ G and  $\gamma$ M cells in antituberculin antibody response, the regional lymph nodes and the spleens from rabbits injected with tuberculoprotein were studied immunofluorescently.

$\gamma$ G and  $\gamma$ M were isolated from rabbit serum. Chickens were immunised with each of these globulins to provide immune globulin. In parallel, rabbits were immunized with chicken  $\gamma$ -globulin to give anti-chicken  $\gamma$ -globulin, which was then labeled with fluorochrome. Tissue slices to be tested were first soaked with chicken anti- $\gamma$ G or anti- $\gamma$ M globulin and, after washing, covered with labeled rabbit anti-chicken  $\gamma$ -globulin.

This method does not always identify specific antibody globulin; It detects also other non-specific globulins relating to  $\gamma$ G or  $\gamma$ M. Plasma cells containing  $\gamma$ G were present in a form of mass in the medullary cords, while cells containing  $\gamma$ M were frequently found in the diffuse cortex around the follicles, their morphology resembling the reticular blast cells or the large lymphocytes. However, whether or not the cells containing  $\gamma$ M represent always the morphology of this kind still remains to be studied.