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Abstracts of "Tuberculosis Research"

Vol. 13, 1960

Immunological Study of Bacillary Constituents of the Tubercle Bacillus

Tadashi KUWAJIMA

Varying protein, polysaccharide and phosphatide fractions isolated from tubercle bacilli and tuberculins were injected into guinea-pigs, at the rate of 1 to 2 mg each, using as adjuvants wax isolated from BCG, paraffin oil and mineral oil (Drackeol) mixed with detergent (Arlacel). The animals were challenged 6 weeks after the injection of the fractions with virulent human type tubercle bacilli. Six weeks after the challenge, all the animals were sacrificed to be examined of their acquired resistance.

When used alone, almost all the fractions showed no appreciable immunizing effect. However, it was worthy noticing that one polysaccharide fraction isolated with urea from the human strain H37 Rv conferred on guinea pigs an appreciable resistance against the virulent challenge, when injected mixed with Drackeol and Arlacel. This immunizing effect was almost not intensified by addition to the polysaccharide fraction with a same dose of tuberculin protein (PPD-s), but it intensified considerably the tuberculin skin reactivity of the animals. Both the immunizing effect and the skin reactivity became more and more pronounced by successive addition of phosphatide and wax to surpass finally the similar effects conferred by injection of the same dose of killed tubercle bacilli.

This result would indicate that tuberculin skin sensitivity (allergy) has not much to do with acquired resistance (immunity), and that a certain sort(s) of bacillary polysaccharide symplex has a significant role in the mechanism of development of acquired resistance against tuberculosis.

Study on the Specificity of the Different Passive Hemagglutination Tests used for Serological Purposes in Tuberculosis

Seiichi FUJITA

Some thirty samples of protein and polysaccharide fractions extracted from tubercle bacilli and tuberculins were tested for their sensitizing ability for both normal sheep erythrocytes and erythrocytes tanned with tannic acid, using Middlebrook-Dubos' and Boyden's tests. It was found that polysaccharide fractions containing less than 1 percent of nitrogen sensitized exclusively normal erythrocyte and protein fractions having less than 1 percent of polysaccharide (Anthrone) sensitized only tanned erythrocytes. Polysaccharide fractions containing more than 1 percent of nitrogen and protein fractions containing more than 1 percent of polysaccharide were found to possess a dual character: they sensitized both normal and tanned erythrocytes. According to the method of extraction, there were some fractions completely deprived of their sensitizing ability for both normal and tanned erythrocytes.

Normal sheep erythrocytes or kaolin particles previously sensitized with one protein or polysaccharide fraction either possessing a single serologic character could absorb its corresponding antibody from tuberculous serum, leaving almost intact its non-corresponding antibody in the serum. The serum suc-

cessively absorbed with protein and polysaccharide fractions gave no longer positive reactions in contact with erythrocytes sensitized with either of the fractions, while its reactivity for erythrocytes sensitized with bacillary phosphatide remained intact.

Study on the Kaolin-agglutination Reaction by the Phosphatide of the Tubercle Bacillus

Hajime FUKAE

It was already reported by Takahashi and Ono from this laboratory that the phosphatide of the tubercle bacillus was well capable of sensitizing normal sheep erythrocytes to hemagglutinations in the presence of tuberculous serum. It was found afterwards that kaolin powder properly heated and finely suspended in deionized water could also be sensitized with the phosphatide to give agglutinations just as high as or higher than hemagglutinations.

In the present experiment, study was made on the conditions of the kaolin-agglutination reaction. In the case of the hemagglutination reaction, the quantity of phosphatide necessary for sensitizing erythrocytes is 0.5 mg/mℓ of saline, while, in the case of the kaolin-agglutination, it is about 0.01 mg/mℓ of saline, just one fiftieth of the quantity necessary for the hemagglutination reaction. Again, in the case of the hemagglutination reaction, the test tubes for reaction must finally be left overnight at room temperature, after they are incubated at 37°C for 30 minutes, in order that the erythrocytes may sink to the bottom of the tube to form clumps, while, in the case of the kaolin-agglutination reaction, the reading can be made by centrifuging the tubes at about 2,000 r. p. m. for 5 minutes, immediately after their incubation terminates.

Studies on the Effect of Repeated BCG Vaccinations upon Experimental Tuberculosis

Masaji TANINO

1) BCG vaccine exhibited an excellent protective activity against experimental tuberculosis in guinea pigs, even when it was injected into the animals definitely infected with tubercle bacilli. In this experiment, repeated BCG injections into guinea pigs infected with virulent human type tubercle bacilli resulted in a marked prolongation of survival time of the animals compared with the non-treated control.

2) The protective effect of BCG vaccine was especially striking when the infective dose of tubercle bacilli was small, while the effect was lowered considerably when the dose was too large. In this respect, BCG vaccination may be expected to be highly effective in the case of natural infection, because in natural infection, the infective dose of tubercle bacilli is generally considered to be far smaller than in the case of experimental tuberculosis.

3) BCG vaccination caused to appear no allergic aggravation in the development of tuberculous infection, and in most of the animals the tuberculin skin-reaction turned negative by repeated injections.

4) In short, this study may serve as an experimental basis for the possibility and the utility of BCG vaccination being used in persons who are tuberculin-positive. Thus, this study will give room for reconsideration of the traditional BCG vaccination which is limited to tuberculin-negative persons.

Pre-operative Conditions and Post-operative Urine Quotients — with special reference to tuberculosis patients —

Yoshiyasu HARADA

Urine samples were taken in the pre- and post-operative stages from 104 patients who underwent pulmonary resection. The urine quotients and urine Na/K were measured thereof, and the measurements were compared with the clinical findings of the patients. The experiments led to the following conclusions.

With the assumption that the O/K_4 method serves as a vitality criterion in human bodies.

- 1) There is no need of consideration for patients who show in the pre-operative stage comparatively low values in the erythrocyte-counting, the pulmonary capacity and the non-breathing time.
- 2) Much precaution should be paid for patients who show already in the pre-operative stage a low value in the eosinophil-counting. This is often the case for persons having a high or low blood pressure, younger and aged persons and persons having high values in the bleeding time.
- 3) Since retardation to normal of the body reactions is often observed in patients who show in the pre-operative stage high Na/K values, salt metabolism should be regulated before operation in these patients.

On the Effect of the Isoniazid-Sulfonamide Joint-Therapy as Seen through the Urine Quotient

Tosuke FUJITA

With an intent to grasp the effect of sulfonamide as a conditioning factor in the tuberculosis therapy, the author selected 93 tuberculous patients and 13 normal adults. Forty-seven patients were subjected to the joint therapy (referred to as I. S. G.: 0.3 g INAH, 3 g Sulfisoxazole per day) and 46 patients were administered INAH alone (I. G.: 0.3 g INAH per day). Periods of administration were 10, 20 and 30 days. Upon termination of each period, INAH-oral-load tests (0.5 g or 1.0 g) were carried out and urine samples were collected 3 times at 2 hour intervals to be measured of their urine quotient (O/K_4).

- A) A follow-up observation after administration of 0.5 g INAH
 - a) Normal adults: O/K_4 -value showed a gradual increase during the period.
 - b) I. S. G. and I. G.: In I. G. group the value decreased in the case of INAH administration, while the value in I. S. G. group showed a temporary decrease immediately after administration.
- B) Period of the joint therapy and INAH load tests
 - a) Ten patients (I. S. G.) were given INAH load of 0.5 g after 10, 20, 30 day periods, respectively. After the 20 day period, a re-rise in O/K_4 -value was not seen, showing a similar curve to that of the above mentioned I. G..
 - b) Nine patients (I. S. G.) were given INAH load of 1.0 g after 10 day period. In this case a re-rise of O/K_4 -value was not seen.

Conclusion :

When the above results are summarized on the basis of the assumption that urine quotient values indicate the state of vitality of the living body, it may be said that in tuberculous patients, the effect of sulfonamide and INAH show an entirely opposing significance from the point of view of the stress concept. In other words, administration of sulfonamide has its significance in enlarging the sphere against INAH-Stress in tuberculous patients.

Influence of the Wax of Tubercle Bacilli on the Sensitizing Activity of Protein

Harue OKUYAMA and Kazuo MORIKAWA

In order to verify the influence of the wax of tubercle bacilli on the sensitizing activity of ovalbumin and tuberculoprotein, the following experiments were undertaken.

Rabbits were sensitized with either ovalbumin or tuberculoprotein mixed with bacillary wax and adjuvant (Arlacel and Drackeol). Seventy or fifty days after sensitization they were re-injected with ovalbumin or challenged with virulent tubercle bacilli, respectively.

At ten day intervals after sensitization the intensity of Arthus' phenomenon and the serum precipitin titer to the respective antigen were measured. The experiments led to the following results:

- 1) The serum precipitin titer remarkably increased by the addition of the bacillary wax to the sensitizing antigen.
- 2) The skin sensitivity also intensified by the addition of the wax. However, the sensitivity of the animals which showed high precipitin titer was rather weak.
- 3) The skin sensitivity provoked by the sensitization with protein mixed with wax was of the immediate type.
- 4) Almost no resistance against virulent challenge could be observed in the animals sensitized with tuberculoprotein mixed with wax.
- 5) The intensity of anaphylactic shock was found to have little correlation with the quantity of the wax used, the precipitin titer and the intensity of skin sensitivity.

Influence of Antituberculous Drugs on Experimentally Produced Tuberculous Pleuritis

Ichiro BAN

The influence of antituberculous drugs on tuberculous pleuritis experimentally produced in guinea pigs was studied histologically.

Guinea pigs previously immunized with BCG, and then challenged intrathoracically with virulent tubercle bacilli showed severe exudative inflammation in the pleura, and lately fibrous lamellar thickening, accompanied with infiltration of small round cells and polymorphonuclears.

However, guinea pigs treated with SM or INAH showed a weaker exudative reaction in the pleura, and no progression of productive changes. In the pleura of the treated animals cell types, which participates directly in the mechanism of the general defence reaction, such as histiocytes, plasma cells and large mononuclears, was found in abundance.

Influence of Prednisolone on the Development of Tuberculous Pleuritis and Pleural Adhesion

Fumihiko NIINUMA

In order to study the influence of prednisolone on experimental tuberculous pleuritis, especially on postpleuritic adhesion, 40 rabbits previously immunized with BCG, and then intrathoracically challenged

with virulent tubercle bacilli suspended in human blood were treated with an 1 or 0.5 mg prednisolone on alternate days.

At two week intervals after challenge they were sacrificed in turn group by group, to be histologically examined of their pleural lesions.

In the early stage a severe exudative inflammatory lesion was observed in the pleura of non-treated control animals, more or less exudative fluid being found in the pleural cavity. The fluid reached a volume of even 70 ml in one case.

In the later stage fibroblastic proliferation became so pronounced in pleural lesion as to develop fibrous adhesion, just as seen in human pleuritis.

On the other hand in the animals treated with prednisolone exudative fluid was scanty and almost no pleural fibrous adhesion could be found. Histologically, prednisolone remarkably inhibited the exudative process and the differentiation of monocytes into epithelioid cells.