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PREPARATION OF IMMUNE BLOOD, SEPARATED PLASMA AND SERUM OF SAID BLOOD

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This invention relates to improvements in immunization of human body against infectious diseases, and is specially directed to the preparation of immune blood, separated plasma and serum of said blood.

The primary object of the present invention is to provide the blood for transfusion, separated plasma and serum for use of treatment and prevention against infectious diseases of human beings, especially against tuberculosis.

Another object of the invention is to produce tuberculosis antigens for use of treatment, prevention and immunization training of tuberculosis.

Numerous other objects and advantages will become apparent in the course of the ensuing description.

The following is the principle of my method described in regard to tuberculosis as prototype.

According to the ordinary accepted ideas on tubercle immunity, tuberculosis does not establish remarkable active immunity, since it is known that tuberculosis recurs, much less in regard to the passive immunization, there can exist, though the group of accessory immunity reactions (agglutination and complement binding reaction, etc.) very weakly exists, but the principal immunity, reactions against tubercle bacilli and their products.

But it is an error due to the circumstances that the high-grade individual differences of human beings were neglected and so the observations were summarized only on diseased or half-diseased persons, except the experimentation on animals.

And now diseased and half-diseased persons are constitutionally susceptible to the infection, namely have individuality not to produce immunity, and the more deviated from the standard, the more susceptible and the more inclined to aggravation. And I have discovered that it is possible to reverse this conclusion and proved this experimentally as follows.

I selected persons having individuality of potent immunity producing faculty and carried out successive gradational immunization training by inoculating on them tubercle bacilli and their products. By this method I succeeded in producing a high-valued active immunity. And then in the use of human blood, plasma and serum thus obtained, there is no limitation in application as in the case of animal-sera, because there is no fear of provoking serum sickness by injection of alien proteins of therapeutic animal-sera. So I could not only use very great doses of high-valued antigen, but also produce such high-valued immune bodies as this invention which never have been obtained heretofore in animals except in human bodies.

The above-mnetioned theory and discovery relating to tuberculosis can be extended to various other infectious diseases, besides tuberculosis, as will be experimentally demonstrated. And the invention rests on this theory and discovery.

And I describe in detail first the application of this method to tuberculosis.

Preparation of Tubercle Antigen

A. Powerful soluble tubercle bacilli antigen

The process of preparation is as follows. Being fished with a spiral large platinum loop a live colony of a tubercle bacilli strain of fixed virulence (glycerine bouillon culture of a strain of powerful fixed virulence of Doctor Shuzo Sato, the Institute for Infectious Diseases, Imperial University of Tokyo), being removed then the rest of the culture medium on a sterilized blotting-paper, being added on it 0.1% chlor-calvacroled ether-alcohol or 0.1% chlor-calvacroled acetone water of the same weight as the bacilli, being dispersed then the bacilli by stirring, being stored up in a air-tight glass container, frequently shaken, kept therein over seven days, being afterwards centrifuged, a supernatant yellowish fluid is obtained, which is referred to as the Solution I and retained air-tightly. After being dryed the said bacilli mass, being weighed and ground in a porcelain mortar to fine powder, being then tested to see under a microscope if the bacilli lose its rod-forms, take indefinite gallerte-forms and lose acid-fastness, being thereupon again weighed and measured to see the decrease by scattering during the grinding, being then again added 0.1% chlor-calvacroled physiological solution, being kept in air-tight container at 37° C., frequently shaken over twenty hours and being afterwards centrifuged, a supernatant fluid is obtained, which is referred to as Solution II and retained air-tightly. Precipitated bacilli mass being again ground, being added on it 0.1% chlor-calvacroled 40% acetone water and infused, being again centrifuged, and a supernatant fluid is obtained which referred to as Solution III, the precipitate is discarded.

The retained three supernatant fluids being mixed, filtered through sterilized filter-paper, yellowish a little turbid fluid is obtained. This fluid is referred to as the Powerful Soluble Tu-

bercle Bacilli Antigen (abbreviated in the following as antigen A). 1 cc. of this fluid contains 0.035 gr. of the above-mentioned tubercle bacilli in raw state. This original solution 1 cc. is calculated as 10,000 units of this antigen by use.

As this antigen contains no formed material, when subcutaneously inoculated after having been adequately diluted (for example 10,000 times diluted with physiological solution), it is easily resorbed and is able to mobilize the faculty of producing antibodies of the tissue cell of the whole body and particularly of blood forming organs, the chief immunity producing organs. And moreover as it stays little at the inoculated place, accessory actions, putrefaction, etc. can be avoided.

Antigen A is, as in the following described, chiefly used for the purpose of high-valued tubercle immunization, but also applicable as such for therapy and prophylaxis of tuberculosis.

And the therapeutic application of antigen A as such on acute, sub-acute and chronic phthisic forms is contra-indicated, as the antigen unpreparedly applied, is liable to aggravate the disease on account of its potent virulence. In such circumstances, before the application of antigen A, the disease focus is cured or disease process is suppressed completely or relatively by use of tubercle immune blood and serum (products afterwards described in this specification) which is obtained by inoculation of tubercle bacilli and their products (chiefly antigen A) into persons of individuality to be highly immunized; thereupon active immunizing faculty is quickened to increase by inoculation of antigen A on the good opportunity.

But on the contrary the chronic non-phthisic type is in many cases cured by amelioration of the general state and suppression of symptoms through repeated inoculations of this antigen.

In use I provide a 10,000 times diluted solution of this antigen. For adult persons inoculation dose begins with 2 cc., and after disappearance of the reactions doses are successively increased. For the purpose of therapy it is repeatedly applied until cure is achieved. For the purpose of prophylaxis of tuberculosis the inoculation of final dose 0.05–1.0 cc. of the original solution per 50 kg. body-weight suffices.

For children dose is decreased in proportion to body-weight.

B. Modified soluble tubercle toxin antigen

The process of preparation is as follows. A colony of the tubercle bacilli culture is heat-sterilized and then dried and in a porcelain mortar, or in the wet state in a refrigerated mortar ground to fine powder, in both cases until the bacilli lose acid-fastness in staining; then being added on it 0.2% formoled physiological solution, it is kept in a thermostat at the temperature of 37° C. frequently shaken about a week. By this procedure the virulence is modified and stimulating power is decreased. Then it is subjected to filtration and a yellowish transparent fluid is obtained, which is referred to as Modified Soluble Tubercle Toxin Antigen. 1 cc. of this fluid contains 0.005 gr. of dried tubercle bacilli. Antigen B is chiefly used, as in the following description, for the purpose of selecting human beings fit for the immunization training. But also it can be used as such to the purpose of treatment and prevention of tuberculosis.

In use for subcutaneous application antigen B is diluted 2,000 times with the physiological saline solution. For adult persons beginning with a dose of 0.02 cc. doses are increased successively. Tubercle bacilli grow very slowly, different from viri of another acute infectious diseases. And by the inoculation of sufficiently resorbable tubercle toxin on bacillus-keeper of tuberculosis, being avoided local retentive reaction, that is making of pseudo-tubercle focus, when the dose is adopted so as to avoid the stimulation, antitoxin is produced in short times without or almost without stimulating the focus. And antigen B is very easily resorbed, so that the inoculated place shows very rarely retentive reaction. So it has advantage of increasing the faculty to produce active immunity of blood forming organs and tissue cells of the whole body. The use of the antigen B, very attenuated toxin, do no harms on human beings. Therefore antigen B is used for the purpose of producing tuberculosis basal immunization (low active immunization) on children and on persons who cannot tolerate antigen A. Antigen B is inoculated subcutaneously for the purpose of decolorization of skin as it has a powerful remarkable and durable lytic action on pigment in skin cells. And it can be also used as a cosmetic or skin remedy to let go into the skin by applying on skin being mixed with various bases (for example cream, oil, alcohol, aquatic solution and the like).

SELECTION OF HUMAN BEINGS FIT FOR IMMUNIZATION TRAINING

(*Basal immunization training*)

To select human being fit for immunization training, a very diluted solution of antigen A is satisfactory, but to avoid the dangers possible by sensible constitution antigen B, more non-stimulating and more resorbable, is at first fitted.

Thus for the purpose of selecting human bodies provided with individuality of immunity producing faculty, healthy bodies who have no anamnesis of tubercle, are subjected to repeated subcutaneous inoculations of antigen B or A in accordance with immunity producing faculty of each person. And in the course of these inoculations the intensity of possible immunity producing faculty is observed. And only to the bodies of potent immunity producing faculty successive inoculations are repeatedly continued, and they are made blood-givers.

POTENT TUBERCLE IMMUNIZATION TRAINING OF BLOOD-GIVERS

Above mentioned selected persons are inoculated with antigen A or B of successively increasing doses. And the each next dose are determined in consideration of the immunity producing faculty which is known from local swelling, redness, disorder, weakness and fever, etc. Persons with allergic phenomena by a little increase of dose have little possibility for continued potent training.

On persons with no reactions, namely anergic state, doses can be increased rapidly in one or two days. (In such cases also according to the individual differences of immunity producing faculty of concerned persons it turns finally allergic at a certain stage. Then we ean make it either anergic or allergic by mere consideration of the subsequently inoculated dose.)

Thus continuing the inoculation we can make produce in the body tubercle immune body fluid of relative potency which is calculated by immunization ratio, the ratio of inoculated dose divided by body-weight. I use the quotient of the

final dose divided by body-weight (kg.) as unit of immunization.

The verification of the immunity value is made in vitro and the value is known by the principal immunization reaction (effect of the sensibilization) and the accessory immunization reactions (complement fixation test, agglutination, and the like) elevated parallel with high valued training.

One of the tubercle immunization trained blood-givers is a man of body-weight 56.0 kg. who can tolerate the antigen dose 1,250,000 units and its immunization ratio amounts to 22,000 units, which corresponds to 4.375 gr. of live tubercle bacilli.

According to the heretofore accepted tubercle immunity theories, it is considered impossible repeated inoculation doses in series as ar^n, increasing geometrically, but only possible in series 1, 2, 3, 4, 5, . . . or 2, 4, 6, 8, . . . increasing arithmetically, and it is out of the question to inoculate so much virulent tubercle bacilli as this invention demonstrates. As to the N. T. Koch 0.5 mg. or 1.0 mg. was considered as the maximal dose by the most investigators.

Now the tubercle immunity once established by this invention retains its value long lasting.

Then the immunized blood produced in the above-mentioned procedure is drawn for example everyday 100 cc. and is as such transfused into tuberclous diseased or its separated plasma or serum is injected. And in case that the separated blood-corpuscles of the drawn blood is returned to the blood-giver, the repeated drawing of over 100 cc. every day makes no remarkable depression of immunity, which is known practically by lack of the reactions by antigen-inoculation. Observations made on numerous blood-givers show for years no signs of disorder.

I resume the virtue of the tuberculosis immune blood of this invention, which has been so far practically proved, as follows. Incipient pulmonary phthisis in the stage of apical catarrh and hilus adenitis can be in 3 or 4 days by injecting about 3–4000 immunity units, and in the second stage non-open phthisis in 6 or 7 days by injecting about 6–7000 immunity units usually suppressed; especially being applied to acute but non-destructive forms in swelling hyperaemic stage the grave symptoms disappear very rapidly and cure is achieved, so in cerebral meningitis, so in peritonitis, periostitis, nephrotuberculosis, laryngeal tuberculosis, intestinal tuberculosis, and the like; and in the case of phthisis in the third stage, owing to the tissue destruction the percentage of cure sinks under 70%, but patients of incipient and second stage can be almost all cured in short days, being allowed mild activity of life.

Some examples will make apparent the nature of the invention more clearly.

Example 1.—On a person of 52.0 kg. body-weight antigen B is inoculated in 67 days in the successive gradational doses of 1, 2, 10, 50, 250, 1,000, 5,000, 30,000 units, every time almost without reactions. And by the inoculation of 150,000 units allergic state with swelling, redness high fever and the like appeared. By this time 67 days were required and the calculated immunity value amounts to 2,884 units.

After 2 days begins the blood-giving. And in about one month under the condition of returning blood-corpuscles of 445 cc. blood-drawing 13 times, the sum total 1,160 cc., was made. After that antigen B 400,000 units, was inoculated. This time very intense reactions: hypertemperature, swelling, redness continued several days, but gradually disappeared. By this time the calculated immunity value amounts to 7,692 units.

After 10 days blood-giving begins again and under the condition of returning the blood-corpuscles of 1,645 cc. in about 80 days blood-giving amounts to 3,785 cc. Thereupon follows inoculation of antigen A 600,000 units. Since this time the increasing ratio was 1.5, reaction was more insignificant compared with the former time. By this time the calculated immunity value amounts to 11,538.

After 5 days commenced again blood-giving and in about 2 months under the condition of returning blood-corpuscles of 1,955 cc. the sum total amounts to 6,060 cc. And thereupon after about 40 days being inoculated antigen A 1,020,000 units, reaction was the same as the former time. Thus the final calculated value amounts to 19,615 units.

After that in about one year every month 1,000–2,500 cc. blood was drawn without any signs of breaking down.

Example 2.—Being on a person of 72.0 kg. body-weight antigen A. 0.2 units is inoculated, swelling and redness remained over several days and on account of fever business was suspended one day. Next after 7 months by the inoculation of 0.4 units reactions were the same as the former time. After 10 days the dose of the third inoculation was 0.4 units with insignificant reactions, feverless. But after 6 months 0.6 units were inoculated with serious reactions, at last the training was given up.

Some examples of clinical experiences are as follows.

Example 3.—A boy of 16 years, body-weight 33.8 kg., both sides pleurisy, pulmonary infiltration of the second stage, light nephrotuberculosis, light fever, light cough, both sides chest half dull, diminished vesicular breathing, on the light kidney spontaneous and oppressive pain. By transfusion of 800 units of the tuberculosis immune blood, renal pains disappeared promptly in 5 hours. Thereupon the serum or blood were injected 7 times in a week, body weight gained to 37.9 kg., and symptoms disappeared. (Here I use the product of drawn blood (gr.) multiplied by immunization ratio of said blood as the unit of blood for transfusion.) Inoculation of antigen B was made and continued. In 10 days body-weight gained to 43.5 kg. and in 2 months by 11 times' inoculation 3,000 units became tolerable.

Example 4.—Girl of thirteen years old, tuberlosis peritonitis, right apical infiltration, laryngeal tuberculosis suspended, 38–39° C. fever continued, diarrhoea, appetite very poor, distension of belly, umbilical circumference 78 cm., laparotomy was indicated by the physician in charge as the only remaining way, but as the operation can provoke the chest symptoms, bad prognosis was pronounced. This patient was treated every day with the blood or serum of this invention. By the first transfusion all symptoms became milder, and after 5 treatments the umbilical circumference decreased to 66 cm., fever sank to ca. 37° C., after 8 treatments umbilical circumference and temperature became normal. And thereupon followed 4 treatments every other day, body-weight gained to 37.2 kg. All symptoms were suppressed. So the antigen B was inoculated. After the sixth inoculation with 100 units abdominal pains and diarrhoea appeared again, but after the applica-

tion of tubercle immune serum 1,330 units, the next day pains and diarrhoea ceased.

Although I have illustrated so far this invention in connection with tuberculosis, but it may also be applied to the other various infectious diseases, than tuberculosis, of which the existence of immunity is known. Namely by means of inoculating the viri of various infectious diseases and their products as antigen in gradational increasing doses into human bodies selected for high valued immunization training in the course of basal inoculation training, there can be produced in the body fluid passive immune bodies and thus prepared blood, plasma and serum for transfusion for therapeutic and prophylactic use against each corresponding disease.

One of the experiments on other infectious diseases of human beings than tuberculosis is as follows.

Example 5.—A person who had once typhoid fever and is always insensible to vaccine. To this person antigen B was inoculated in 24 months 10 times, beginning with 0.5 units. But finally only 70 units were inoculated with light fever and putrefaction. Thus the faculty of producing tubercle immunity manifested itself relatively low. And the immunization training was given up. Thereupon typhoid bacilli immunization training with typhoid antigen (officially indicated initial dose 0.5 cc., 2nd dose 1.0 cc. for adult) was carried out, beginning with 1.0 cc., in 9 months 10 times, the final dose was 80.0 cc. at one time.

Although I have described my invention setting forth specifically the use thereof in immunization against tuberculosis, it will be apparent from the general nature of the above description that it is intended not to be limited to tuberculosis. From work already done it is apparent that the principles here involved are adaptable to the treatment of many other infectious diseases, of which the existence of immunity is known, with equal advantages and from such work, it is believed that this invention is applicable to practically the entire field of immunization where gradational successive increasing doses of antigen can be used.

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