# PASSIVE TRANSMISSION OF DELAYED TYPE HYPERSENSITIVITY REPRODUCED BY SENSITIZATION WITH STREPTOCOCCAL ALLERGEN

(UDC 616.981.21-097.3)

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(Presented by Active Member AMN USSR, L. A. Zil'ber)
Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 60, No. 12, pp. 62-66, December, 1965
Original article submitted April 3, 1964

According to many investigators, the delayed type of hypersensitivity (DTH) is a type of immune response which is due not to antibodies circulating in the blood, but to the so-called transfer factor, a specific substance of an unknown origin present in the lymphocytes. Most workers have been unable to transmit DTH passively by serum, whereas passive transfer has been carried out successfully using lymphoid cells from lymph nodes, spleen, and peritoneal exudates from experimental animals, and by peripheral blood lymphocytes in the case of man [5,7,9,10,13-15,20]. The significance of small lymphocytes in DTH has been established [21]; it has been demonstrated that the sensitized cells migrate to the site of injection of specific antigen [16]. However, the latter contention is disputable [11, 12].

In previous studies [2,3], it was demonstrated that guinea pigs receiving streptococcal allergen in Freund's adjuvant develop reactions of the delayed type following a second intradermal injection of specific allergen. Since the delayed reactions persisted for a prolonged period, and since, despite the injection of large doses of allergen, no fast reactions due to intensive production of antibodies were observed, it was proposed that the streptococcal allergen leads to a relatively weak production of antibodies, but retains the property to induce DTH.

It is of considerable importance to clarify the problem pertaining to the peculiarities of the antigenic substances from different microbes, which, like tuberculin, lead to persistent unaccompanied-by-fast reactions in several chronic infectious processes. In this connection, it was necessary to confirm that the phenomena which were described in the former communication were not due to circulating antibodies but to specific sensitization of lymphocytes.

In the present work, an attempt was made to bring about experimental passive transfer of increased sensitivity to streptococcal allergen by serum and by lymphoid cells.

## MATERIALS AND METHODS

Streptococcal allergen (thermostable fraction) was obtained according to Verzhikovskii's modification [1] of Ando's method [5]. Guinea pigs (250-300 g) were sensitized by foot-pad inoculation of streptococcal allergen (250-400 micrograms of protein, according to Lowry) with an equal volume of Freund's adjuvant. Only adjuvant was introduced into the control animals. Skin tests were carried out at different periods after sensitization of animals, using 2-4 micrograms of allergen protein.

For passive transfer, we used as donors animals with definite reactions of delayed hypersensitivity 7-8, 15-17, 23 and more days after sensitization (these reactions could be detected macroscopically more than 6 h after intradermal injection of the allergen and reached a maximum by the 24th h). The skin reactions were absent in control animals.

TABLE 1. Results of Passive Transfer of Increased Sensitivity by Lymphoidal Cells and Serum

| Time after sensitization of donors (days) | Lymphoid cells | | Serum | |
|---|---|---|---|---|
| | number of donors | number of recipients | number of donors | number of recipients |
| Experiment (allergen + adjuvant) | | | | |
| 7-8 | 30 | 7/10 | 19 | 0/5 |
| 15-17 | 14 | 2/3 | 39 | 0/8 |
| 23 and later | 10 | 4/4 | 73 | 2/26 |
| Total | 54 | 13/17 | 131 | 2/39 |
| Control (adjuvant + physiological solution) | 17 | 0/6 | 14 | 0/3 |

Remarks: numerator – number of recipients with positive results of transfer; denominator – total number of recipients.

After testing the skin reactions, we used heart blood as source of serum for passive transfer and determination of antibodies. The antibodies were determined by passive haemagglutination and passive skin anaphylaxis. The method of determination of antibodies in-vitro was described in our previous studies [3,4].

Lymphoid cells for passive transfer were obtained from lymph nodes and peritoneal exudate. Ten ml of medium No. 199 was inoculated intraperitoneally and after massaging for 5-7 min, an exudate was obtained, centrifuged for 3 min at 800-1000 r.p.m., and the deposit washed with Ringer's solution. In counting the exudate cells stained with trypan blue in Goryaev's chamber, an average of 61.7% were mononuclear in nature. The lymph nodes (superficial and deep seated inguinal and axillary) were freed of fatty material and were carefully cut up with scissors and suspended in Ringer's solution. Ten min after the heavier particles had settled, the supernatant material was collected, centrifuged, and the sediment was washed with Ringer's solution to which was added 10% by volume of heparin.

Serum of 4-5 animals was mixed and 4-5 ml was introduced intravenously into a guinea pig. The skin tests were made within $1\frac{1}{2}$ h and the results were read after 30 min, 2, 4, 6, and 24 h. The reactions showing erythema more than 5 mm in diameter were considered positive. The suspension of lymphoid cells obtained from 3-4 guinea pigs was mixed and injected intraperitoneally. Each test was made 24-48 h after passive transfer. Reactions were read in the same manner as in the case of serum injection.

## EXPERIMENTAL RESULTS

The main part of the experiments was carried out with lymphoid cells obtained from lymph nodes. The latent period * was 48 h, since it had been found that injection of leucocytes obtained from the exudate and testing after 24 h have the worst results.

Serum from 131 donor animals was injected into 39 recipients to effect passive transfer (Table 1).

The recipients showed skin reactions (weakly expressed) in only two cases – where the animals had been injected with a mixture of sera obtained from animals on the 27th day after sensitization. A suspension of lymphoid cells from the lymph nodes of 54 donors was introduced into 17 guinea pigs. The delayed-type reaction was observed in 13 cases after intracutaneous injection of the allergen. The passive transfer gave positive results regardless of the post-sensitization period used for obtaining the lymphoid cells. In control experiments, negative results were obtained on introduction of either serum or lymphoid cells from animals injected only with the adjuvant.

*Latent period – from the time of introduction of lymphoid cells to the recipients to the intracutaneous injection of antigen into these animals.

TABLE 2. Results of Passive Transfer of Increased Sensitivity by Lymphoid Cells and Serum and Antibody Levels in Donor Sera

| Results of passive transfer | Lymphoid cells | | | | | | | Serum | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | number of recipients | antibodies in donors | | | | | | number of recipients | antibodies in donors | | | | | |
| | | passive anaphylaxis | | | passive hemagglutination | | | | passive skin anaphylaxis | | | passive hemagglutination | | |
| | | – | + | ++ | – | 1:100 – 1:400 | 1:800 and above | | – | + | ++ | – | 1:100 – 1:400 | 1:800 and above |
| | | number of donors | | | | | | | number of donors | | | | | |
| Positive | 13 | 41 | 3 | 2 | 15 | 4 | 22 | 2 | 2 | – | 8 | 5 | 3 | 2 |
| Negative | 4 | 18 | – | – | 7 | 1 | 10 | 20 | 51 | 8 | 16 | 27 | 11 | 33 |
| Total | 17 | 59 | 3 | 2 | 22 | 5 | 32 | 22 | 53 | 8 | 24 | 32 | 14 | 35 |

Remarks: + weak skin reaction; ++ intense skin reaction.

A comparison of the results of passive transfer by serum and by injection of lymphoid cells, and the levels of antibodies in the blood of the donor animals, are given in Table 2.

In those cases when the passive transfer of serum gave a positive result, antibodies were detected in the sera of most of the donors (in 8 out of 10) by the method of passive skin anaphylaxis. In the case of negative results, obtained in passive transfer of serum, only in 24 out of 75 donors, i.e., in one third of the cases, were antibodies found by the method of passive skin anaphylaxis. Such distinct ratios were not detected when antibodies were studied in both groups of donors by the method of passive hemagglutination. In an investigation of the sera of animals from which lymph cells were taken, no significant differences were noted in the antibody level in groups of pigs whose lymphocytes gave positive or negative results in the case of passive transfer (see Table 2).

Thus, the attempt to passively transfer increased sensitivity to streptococcal allergen by large volumes of serum led more often to negative results and only 2 animals, which received serum obtained on the 27th day after sensitization, showed weak skin reactions. On the contrary, the passive transfer of increased sensitivity by lymphoid cells led in most cases to positive results.

Our data agreed with the studies in which it was found that DTH could not be transferred by serum. Hence, the role of circulating antibodies can be ruled out in view of the negative results obtained in the attempt to bring about passive transfer with large amounts of serum. In addition, as has been shown in the previous studies [2,3], the development of skin reactions does not depend upon the presence and level of circulating antibodies. One should eliminate the possibility of active sensitization of the recipients with the antigen which may be transferred with lymphoid cells, since the skin reactions were obtained a short time after passive transfer. The present studies, as is the case with all experimental studies in which there is demonstrated a passive transfer of DTH by lymphoid cells, obtained from lymph nodes, do not eliminate the possibility of the presence of attached antibodies, which may be transferred from the donor together with the cells or be produced by them after transfer.

As for the role of antibodies attached to lymphoid cells in DTH, according to most investigators this involves not the conventional antibodies (immune $\gamma$-globulin), but a special substance (the transfer factor), which has the properties of a specific antideterminant [15,18]. The proof of the latter lies in the possibility of inducing DTH in hypogammaglobulinemia cases, which lack the property of producing antibodies, and, on the other hand, the impossibility of active development of DTH in those ill with Hodgkin's disease and Boeck's sarcoid which can still give skin reactions in the case of passive transfer by lymphoid cells and can still produce antibodies [8,17]. The data indicating the absence of $\gamma$-globulin attached to the lymphocytes, also proves that the transfer factor is evidently a special substance, which differs from the usual immune $\gamma$-globulin [19].

The data obtained in the present study confirm the previously held position that streptococcal allergen, introduced into experimental animals, even in a large dose, does not bring about production of large amounts of circulating antibodies, whereas DTH develops. It should be pointed out, that in the above indicated case, no special methods were employed which are usually used for suppression of antibody production when different proteins are injected into experimental animals to induce DTH (introduction of antigen in the form of precipitates, obtained in the zone of antibody excess, or as conjugates with chemical haptens). The above data form the basis for assumption that antigenic substances differ in their ability to stimulate antibody formation and DTH.

Thus, it is possible to passively transfer increased hypersensitivity to streptococcal allergen in experimental animals by introduction of lymphoid cells and that, in most cases, it is not possible to do this using large volumes of serum.

Our data demonstrate that the increased susceptibility brought about by streptococcal allergen must be classified as DTH.

The attainment of long-lasting, delayed-type hypersensitivity by injection of large amounts of streptococcal allergen without the use of special methods to suppress antibody production indicates some special features of certain substances of bacterial origin.

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All abbreviations of periodicals in the above bibliography are letter-by-letter transliterations of the abbreviations as given in the original Russian journal. *Some or all of this periodical literature may well be available in English translation.* A complete list of the cover-to-cover English translations appears at the back of this issue.

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