A STUDY OF THE NATURE OF THE ALLERGY TO STREPTOCOCCUS IN RHEUMATISM

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Rheumatism is regarded by most authors as an infectious-allergic disease. The prevalent point of view in the current literature is that sensitization in rheumatism is caused by streptococcal sensitizing agents. N. D. Strazhesko considers tonsillitis as a primary cause creating favorable conditions for the development of rheumatism. During tonsillitis, the organism reabsorbs the products of streptococcal growth from the tonsils, causing sensitization against the background of which rheumatism develops. From this, the constant pathogenetic connection between tonsillitis and rheumatism, which is documented by a large number of observations, becomes understandable. According to N. D. Strazhesko, tonsillitis precedes rheumatism in 64% of the cases, according to A. N. Koritsky, V. L. Bisyarina and M. G. Mirkina, in 70-80%, while according to E. N. Nezlin, in an overwhelming majority of patients, given good recollection.

Starting with 1948, evidence has appeared on the importance of blood cells in adsorbing sensitizing substances in various allergic states. A similar fact was initially stated by Chase [4] for tubercular allergy. This author established that in persons who react positively to tuberculin, washed leucocytes from peritoneal exudate sensitize guinea pigs. Following the intracutaneous injection of the latter with tuberculin, they develop a hyperallergic reaction. In 1952, H. Lawrence reported on the sensitization of healthy volunteers who had been injected intracutaneously in the shoulder region with washed leucocytes of persons having a positive reaction to streptococcal allergen. As a result, they developed passive allergy to streptococci, lasting from 1 week to 3 months. On the basis of these experiments, H. Lawrence concluded that leucocytes from people who had earlier suffered streptococcal infections, adsorb substances "resembling tuberculin," i.e., streptococcal sensitizing agents.

The investigations of Chase and Lawrence were the premise for our investigations. We set ourselves the problem of determining whether the leucocytes and erythrocytes of rheumatic patients with positive reactions toward streptococcal allergen adsorb substances of the streptococcal sensitizer type.

EXPERIMENTAL METHODS

Blood was taken from rheumatic patients with strongly expressed allergic reactions to intracutaneous administration of streptococcal allergen and was placed in test tubes containing sodium oxalate (0.01 ml of a saturated solution per 1 ml blood). The contents of the test tube were thoroughly mixed and centrifuged with

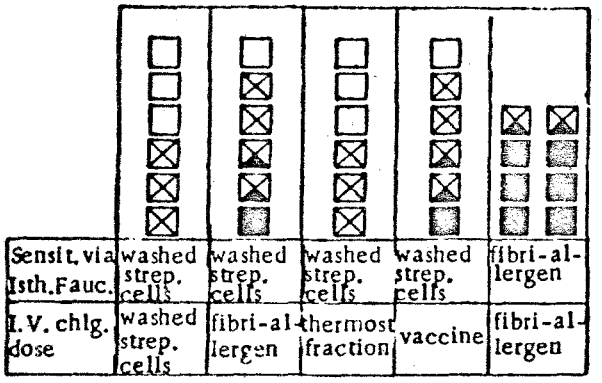

Fig. 3. Results of experiments in which guinea pigs were sensitized with washed microbial cells of the streptococcus in order to determine the presence of sensitizing fractions of the streptococcus in the microbial cells or in the products of its growth, contained in the filtrates of 6-day broth cultures. Designations same as in Figs. 1 and 2.

We set up experiments in which guinea pigs were sensitized with washed cells of a hemolytic streptococcus. For the challenge dose, one group of these animals was injected with washed streptococcal cells, another with streptococcal allergen, a third with the thermostable fraction of allergen prepared by us, and a fourth with streptococcal formol-vaccine. The results of these experiments showed that the use of washed streptococcal cells as sensitizing and challenge doses gave a very weak effect; only half of the guinea pigs showed light anaphylactic manifestations. Only the use of allergen and streptococcal formol-vaccine as the challenge dose caused death in one of each six pigs; two of each group had a severe form of anaphylaxis, and two had a light form of anaphylaxis (Fig. 3).

Such an effect could only be caused by substances from 6-day broth cultures of streptococci which pass through a bacterial filter (F_2). These substances are apparently thermolabile, since the thermostable allergen fraction used as the challenge dose had a weaker activity as did the washed streptococcal cells. When the results of sensitization with washed streptococcal cells and the thermostable allergen fraction are compared with the sensitizing activity of the thermoabile fraction of the allergen, the great sensitizing activity of the latter becomes immediately apparent.

Therefore, in rheumatism it is not the microbial cells of streptococcus themselves that sensitize, but rather the products of their growth, which pass through bacterial filters and are apparently thermolabile. These substances may be either fragments of microbial cells or filtrable forms of the streptococcus.

The facts presented lend new support to the concept of the streptococcal nature of rheumatism. Evidently, allergy to streptococci in rheumatic patients is, as a rule, accompanied by the presence of streptococcal sensitizing agents adsorbed to the formed elements of the blood. Regardless of how we regard these sensitizing agents, either as disintegration products of microbial cells or as filtrable forms, we have a new fact, proving the systematic presence of streptococci in the organism of rheumatic patients. Also, on the basis of O. V. Krasovskaya's work [2, 3], who used the method of staining blood smears of rheumatic patients according to Morozov and electron microscopy there are serious reasons to consider these formations as filtrable forms of

repeated washings with physiological saline and aspiration of the upper layer, containing leucocytes. The sediment, consisting of erythrocytes, was also subjected to washing with physiological saline. As a result, washed leucocytes, on the one hand, and washed erythrocytes, on the other were obtained.

In one set of experiments, we injected guinea pigs with washed leucocytes in the region of the isthmus focium,* in another, washed erythrocytes were injected, while in a third, a mixture of leucocytes and erythrocytes. The sensitizing dose was equal to 0.2-0.4 ml. In 3 weeks, the guinea pigs were injected intravenously with a challange dose of streptococcal allergen (filtrate of 6-day broth cultures of a hemolytic streptococcus). Controls for this investigation were experiments with sensitization of guinea pigs with streptococcal allergen and with the intravenous injection of a challange dose of the latter. In addition, there were animals in the control groups into which broth was injected as the sensitizing and resolving doses, and also washed leucocytes and erythrocytes from the blood of healthy people who did not react to streptococcal allergen. In all, there were 49 guinea pigs in the experimental group and 50 in the control groups.

EXPERIMENTAL RESULTS

Experiments in which 14 guinea pigs were injected with washed leucocytes in the region of the isthmus fociumand subsequently injected intravenously with streptococcal allergen gave rise to anaphylaxis in 7 of the animals. The most interesting experiments were those on 9 guinea pigs, injected with washed leucocytes from Patient D, who had a marked hyperallergic reaction to streptococcal allergen; 4 of the guinea pigs showed typical anaphylaxis, while washed erythrocytes from the same patient evoked mild anaphylactoid symptoms in 2 guinea pigs. However, when leucocytes were used in a mixture with erythrocytes, one of 3 guinea pigs developed severe anaphylaxis, terminating in the death of the animal, while the other 2 guinea pigs showed light signs of anaphylaxis. These experiments showed that leucocytes apparently adsorb streptococcal sensitizing agents more intensively than do erythrocytes in the organism of the rheumatic patient. On the other hand, streptococcal sensitizing agents were adsorbed unequally, since, in some animals, anaphylaxis following sensitization and challenge doses appeared with all its inherent manifestations, while only a weak anaphylactoid reaction was observed in others.

Analogous facts were also recorded when washed leucocytes and erythrocytes from the blood of other patients examined by us were used. In a total of 40 guinea pigs, typical anaphylaxis was produced in 19 animals, while anaphylactoid symptoms were observed in 11 pigs (Fig. 1).

In sensitizing guinea pigs with streptococcal allergen the injection of a challenge dose of the same preparation (filtrate of 6-day broth cultures of streptococcus) evoked anaphylaxis in all of the animals, in most cases terminating in death. In order to determine the specificity of action of the allergen, we added serum from guinea pigs which had recovered from anaphylaxis and which were, according to A. M. Bezredky's determination, in a state of antianaphylaxis. After sensitization of the guinea pigs and the injection of the challenge dose of allergen mixed with serum, anaphylaxis developed in only one of the five animals (Fig. 2). Therefore, sensitization occurs due to a specific streptococcal sensitizing agent against which guinea pigs recover from anaphylaxis produce a neutralizing substance in the period of antianaphylaxis.

Control experiments, with the use of broth alone in the capacity of sensitizing and challenge doses, and also with washed leucocytes and erythrocytes from healthy persons, gave negative results; neither anaphylaxis, nor even anaphylactoid manifestations, were produced in the animals in a single case.

On the basis of the data presented, it is possible to conclude that the leucocytes and erythrocytes in the organism of rheumatic patients adsorb specific streptococcal sensitizing agents which cause allergy in rheumatism.

We were also interested in the question of whether sensitization takes place due to whole microbial cells, or due to their products of disintegration (and possibly filtrable forms) contained in streptococcal culture filtrates.

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* On the basis of our work published in the Byull. Eksptl. Biol. i Med. No. 12, p. 48 (1954), it was established that the region of the pharyngeal arches in guinea pigs is the most sensitive to sensitization.

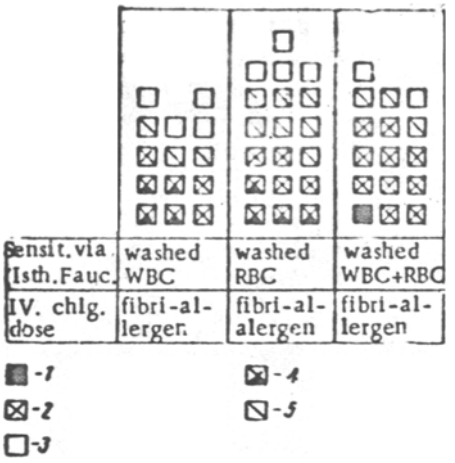

Fig. 1. Results of experiments in which guinea pigs were sensitized with washed leucocytes and erythrocytes from the blood of rheumatic patients with positive reactions to streptococcal allergen, with subsequent injection of the latter intravenously as the challenge dose.
1) Guinea pig succumbed to anaphylaxis; 2) light manifestations of anaphylaxis; 3) no reaction; 4) severe manifestations of anaphylaxis; 5) anaphylactoid manifestations.

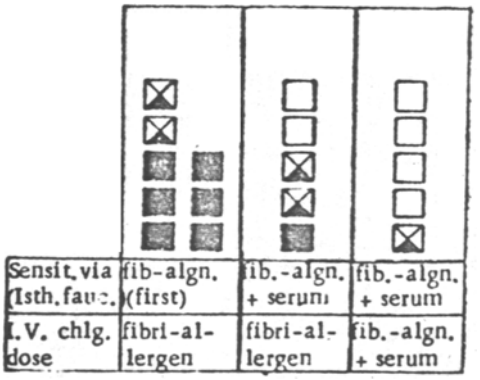

Fig. 2. Results of experiments in which guinea pigs were sensitized with filtrates of 6-day broth cultures of streptococcus and in which the specificity of streptococcal sensitizing agents was determined by the neutralization method using antianaphylactoid serum from guinea pigs which had survived anaphylaxis following sensitization and challenge doses of streptococcal allergen. Designations same as in Fig. 1.

streptococcus. Under the electron microscope, they have spherical or ovoid forms and are 200-700 millimicrons in size. This is confirmed by our investigations on prolonged incubations of inocula from the blood of rheumatic patients in whom O. V. Krasovskaya had found the formations mentioned above; under conditions of anaerobic incubation, cultures of streptococcus grow, frequently having the properties of the anaerobic microstreptococci described by A. Prevot [7]. In addition, growth of streptococcal cultures is found upon prolonged incubation in semisolid agar and on Kitt-Tarocci medium (for growing anaerobes) of blood inocula from rheumatic patients.

On the basis of the experiments conducted, we can consider that washed leucocytes and erythrocytes of rheumatic patients having a positive reaction to streptococcal allergen contain sensitizing substances which produce anaphylaxis to streptococcus in guinea pigs.

Therefore, the data obtained are a new proof of the importance of streptococci in the etiology of rheumatism.

SUMMARY

The problem was studied of the presence of adsorption of streptococcal allergens by the leucocytes and erythrocytes of the patients with rheumatism. The method consisted of sensitization of guinea pigs at the area of arci of the isthmus faucium by washed leucocytes and erythrocytes from the peripheral blood of the patients with rheumatism, with subsequent introduction of the filtrate of old cultures of streptococci to the animals as a booster dose.

As a result of these experiments anaphylaxis was obtained in 26 guinea pigs out of 49, while anaphylactoid reactions occurred in 12. Control experiments showed that the filtrates of old broth cultures of streptococci give the most pronounced sensitization in guinea pigs. These sensitizing substances are neutralized by the serum of these guinea pigs which had anaphylaxis after sensitization and introduction of the booster dose of streptococcal filtrate.

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