A STUDY OF THE TRANSFORMING PROPERTIES OF ALLERGENS

V. N. Polshkova, and P.P. Sakharov

Laboratory of Allergy, Scientific Research Institute of the Ear Nose and Throat, Ministry of Health RSFSR
(Presented by Active Member AMN SSSR N. N. Zhukov-Verezhnikov)
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The theory of transformation developed by microbiologists [5, 6, 10-14], and the study of transduction [15] represent the origin of the modern genetic theory of immunity and allergy.

According to Ehrich's so-called genetic theory of the formation of antibodies "organizer" is ultimately responsible for the formation of antibodies and is produced under the influence of the specific antigen.

Next a hypothesis [20] was advanced concerning the action of antigens on desoxyribonucleic acid (DNA) of cells of the reticuloendothelial system, an action which has the effect that a combination is formed of the antigen with the DNA; the combination then influences the ribonucleic acid present in the cytoplasm to become the producer of the specific antibodies. In line with this view is the evidence that RNA is a supplementary determinant of the antigen and determines the specific synthesis of antibodies [7, 8].

Convincing support for this view has been provided in studies of the nature of the sensitizing properties of washed leukocytes [17].

Because foreign authors have based their genetic theory of immunith and allergy on the concept of a mechanism of transformation we have considered it essential to determine whether allergens exert a transforming influence on strains of bacteria which do not possess the allergen properties of the donor. No such microbiological studies have been carried out.

EXPERIMENTAL METHOD

As donors we used thermostable fractions and filtrates employed as allergens and prepared from six-day cultures of two strains of hemolytic streptococcus (21 and dochez). Strain 21 is classified as a twenty-seventh serotype, and the strain dochez as a tenth serotype; both belong to serogroup A.

As recipients we used streptococcus viridans obtained from a patient with lupus (strain G) and hemolytic staphylococcus (strain 209). These strains have all the characteristic features of streptococcus viridans and of staphylococci and show distinct antigenic differences from the culture of hemolytic streptococcus which we have mentioned. These differences are that the type-specific serum of serotype 27 agglutinated strain 21 to a titre of 1 : 3200 (rating ++++), but did not agglutinate a strain of streptococcus viridans or the hemolytic staphylococcus. Hemolytic streptococci are known to possess a type-specific substance M which is absent from streptococcus viridans and from staphylococci.

We have carried out several sets of experiments in which 50 passages were made of streptococcus viridans strain G through liquid nutritive media; in one set of experiments the medium contained 20% unheated filtrate of strain 21 and in the other a filtrate which had been heated in an autoclave at 1.5 atmospheres for 30 min; in a third set of experiments we used as donor microbial cells of strain 21 killed at 70°; a fourth set of experiments was carried out with the thermostable fraction of strain 21 prepared by precipitation in acetic acid and subsequent neutralization and heating for ten minutes in a boiling water-bath. Similar experiments were made with hemolytic streptococcus grown on the thermostable fraction prepared as described and in media containing killed microbial cells of strain 21.

For the experiment we used 157 guinea pigs of which 106 were twice sensitized with the allergens from hemolytic streptococcus strain 21; 25 animals were sensitized with streptococcus veridans strain G, and 17 with the same pathogen grown on the allergen substances of the hemolytic streptococcus while 11 guinea pigs were sensitized with the hemolytic staphylococcus itself.

Results Obtained in Growing Streptococcus Veridans and Hemolytic Streptococcus on the Thermostable Fraction of Strain 21 and on the Heated Filtrate of Strain 21

Results of experiments	Critical dose					
	strain GC	strain 21C	transformed strain G and heated filtrate of strain 21	transformed strain G from TS fraction of strain 21	Staphylococcus strain 209	transformed strain 209 on TS fraction of strain 21
Number of guinea pigs used in the experiment	21	19	11	17	6	8
No anaphylaxis	3	11	1	2	3	-
Different manifestations of anaphylaxis	18	8	10	15	3	8
Mean index of anaphylactic manifestations	1.8	0.66	1.8	1.64	0.5	1.5

Symbols used: Strain 21 C) hemolytic streptococcus strain 21 (control); strain GC) streptococcus veridans strain G (control); TS fraction of strain 21) thermostable fraction of strain 21; heated filtrate of strain 21) filtrate of culture of hymolytic streptococcus strain 21 heated in an autoclave (1.5 atmospheres) for 40 min; Staphylococcus strain 209) control strain of staphylococcus; transformed strain 209 on TS fraction of strain 21) strain of staphylococcus transformed on the thermostable fraction of hemolytic streptococcus strain 21.

One month after sensitization the guinea pigs received into the blood stream a critical dose of microbial cells washed and killed by heating at 70° for one hour on a water-bath; at the same time they received filtrates of the control and experimental cultures. The number of microbial cells was 2 billion per ml.

EXPERIMENTAL RESULTS

The results obtained are summarized in the table.

The index indicating the degree of anaphylaxis was calculated as follows.

Anaphylactoid phenomena were taken as a unit, a weak form of anaphylaxis 2 units, marked anaphylaxis as 3 units and death from anaphylaxis was represented by 4 units.

According to the number of animals in which a particular form of anaphylaxis was manifested we obtained a total which was divided by the number of guinea pigs used in each group of experiments.

The investigations show that streptococcus viridans and the hemolytic streptococcus grown in the media containing the allergen substances of the hemolytic streptococcus acquired the latter through transformation. The transformation is brought about firstly by transmission of the protein thermostable substance M of the hemolytic streptococcus which as we have explained is entirely absent from control cultures of streptococcus viridans and of the hemolytic streptococcus. A test of type-specific features at the 50th passage shows that the experimental strain of streptococcus viridans (of the recipient) acquired a substance M27 of the serotype of the hemolytic streptococcus in titres 1 : 200- 1 : 800, and did so almost equally in all series of the experiment; in each case as donor we used microbial cells or the heated filtrate or the thermostable fraction of the allergen of the hemolytic streptococcus. Hemolytic staphylococcus cultured under similar circumstances also acquired a substance M, but in smaller titres - 1 : 50 - 1 : 100.

The sensitization of guinea pigs by the strain of streptococcus viridans transformed (on the hemolytic streptococcus) gave a result which differed from that obtained when the sensitization was carried out with the initial streptococcus viridans culture. Sensitization and the injection of a critical dose of the control streptococcus viridans strain induced an anaphylaxis of index 2.03; sensitization with the transformed strain of streptococcus viridans injected in a critical dose of the control strain G was an extremely weak anaphylactic reaction of index 0.82-0.87. If the transformed strain of streptococcus viridans in the last experiment constitutes a critical dose the anaphylactic index is raised to 1.4.

We have thus demonstrated transformation through allergens of allergizing substances of the hemolytic streptococcus to streptococcus viridans and to the hemolytic staphylococcus; this transformation is brought about by a protein

substance M containing no DNA [21]. These results confirm our previous investigations [1, 2, 3, 4], and also the work of many foreign authors [16, 18, 19, 22] showing that transformation of bacteria may be brought about by proteins produced in the vital processes.

SUMMARY

Investigations carried out demonstrated that thermostable and autoclave-heated allergen fractions prepared from the fluid decanted from the hemolytic streptococcus culture transformed the allergenicity of Streptococcus viridans in the hemolytic direction, passing to it the type-specific M-substance of the donor.

Detection of the transforming action of protein substances, precipitated from the decanted fluid by means of acetic acid, filtered through bacterial filters and heated at 1.5 atmospheric pressure for 30 min (which had a destructive effect on the DNA) pointed to determinative significance of the protein antigenic complexes. This leads to a conclusion that in the development of immunity and allergy in animals and human beings the transformation mechanism is the same, the difference being that in the latter case the plasma cells of reticuloendothelial system serve as the recipient.

LITERATURE CITED

1. E. I. Gudkova and P. P. Sakhorov, Byull. Éksper. Biol. (1957), No. 10, p. 81.
2. V. M. Polshkova, Byull. Éksper. Biol. (1963), 4, p. 66.
3. P. P.Sakhorov, E. I. Gudkova, V. B. Bureva et al., Agrobiologiya Byull. Éksper. Biol. (1959), 3, p. 362.
4. P. P. Sakharov, E. I. Gudkova, V. M. Polshkova et al.,Byull Éksper. Biol. (1961), 10, p. 80.
5. H. E. Alexander and G. J. Leidy, J. exp. Med. (1941), 93, p. 345.
6. O. T. Avery, C. M. MacLeod, and M. McCarty, Ibid. (1944), 79, p. 137.
7. F. M. Burnet, Enzyme, Antigen and Virus, Cambridge (1956),
8. Idem, Principles of Animal Virology, New York (1960).
9. W. K. Ehrich, Handbuch der allgemeinen Pathologie, Berlin (1956), 7, p. 1.
10. H. Ephrussi-Taylor, Exp. Cell. Res. (1951), 2, p. 589.
11. R. D. Hotchkis, Unités biologiques douces de continuité génétique, Paris (1949), p. 57.
12. Idem, Cold Spr. Harb. Symp. quant. Biol. (1951), 16, 457.
13. Idem, Phosphorus Metabolism, Baltimore (1952), 2, p. 426.
14. Idem, The Chemical Basis of Inheritance [in Russian], Moscow (1960), 251.
15. F. Jacob and E. Wolman, The Chemical Basis of Inheritance [in Russian], Moscow (1960), p. 376.
16. A. Kleczkowski, Brit. J. exp. Path. (1940), 21, p. 98.
17. H. S. Lawrence, Cellular and Humoral Aspects of Hypersensitive States, New York (1959).
18. J. Marrack, The Chemistry of Antigens and Antibodies, London (1938).
19. E. Rajka, Allergie und allergische Erkrankungen, Budapest (1957), 1.
20. R. S. Schweet and R. D. Owen, J. cell comp. Physiol. (1957), 50, Suppl. 1, p. 199.
21. J. Smolens and H. Warner, J. Immunol. (1952), 68, p. 185.
22. F. Jacob and E. Wolman, The Chemical Basis of Inheritance [in Russian], Moscow (1960), p. 376.
