CHANGES IN THE ANTIGENIC COMPOSITION OF THE LUNGS IN EXPERIMENTAL BERYLLIOSIS

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Six new antigens were determined by immunoelectrophoresis in the nucleoprotein fraction of the lungs of August rats with experimental berylliosis. Their electrophoretic mobility was determined relative to human serum albumen. The use of active anaphylaxis and Zil'ber's method showed that some of the additional antigens probably contain beryllium while others are free from it.

The first investigation of the antigenic changes in the lungs in experimental berylliosis showed that the nucleoprotein fraction of the lungs not only loses some of its antigens, but also acquires new ones not characteristic of the normal lung [2]. However, the methods used by these workers did not allow the individual antigens in the multicomponent system to be identified. Nor was it shown whether beryllium manifests its properties of a hapten in the lungs in berylliosis, although its sensitizing properties have often been described [1, 8, 9-12].

In the investigation described below an attempt was made by using immunodiffusion methods to detect as fully as possible antigens not characteristic of normal tissue, and also, by use of the anaphylaxis method, to determine whether beryllium is present as a hapten in the new antigenic determinants.

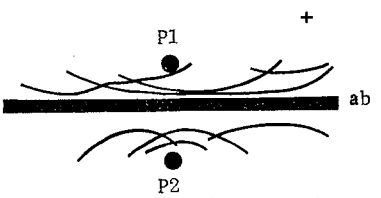

Fig. 1. Diagram showing arrangement of antigens of nucleoprotein fraction of lungs of rats with experimental berylliosis not found in the lungs of intact rats by immunoelectrophoresis. P1) Subfraction of lung nucleoproteins readily soluble in physiological saline; P2) subfraction of the same proteins sparingly soluble in physiological saline; ab) globulins of hyperimmune rabbit serum against nucleoprotein fraction of rats with experimental berylliosis.

EXPERIMENTAL METHOD

Male August rats weighing 100-120 g were used. Experimental berylliosis was produced by the method described previously [4]. The animals were decapitated one month later. The lungs were washed on ice with physiological saline, the root of the lungs was removed, and the remaining lung tissue used for isolation of the nucleoprotein fraction by Belozerskii's method. This fraction was used to immunize rabbits and in agar-diffusion tests [5]. Two subfractions were used in the semimicromodification [11, 6] of analytical immunoelectrophoresis. For this purpose the original nucleoproteins were clarified by centrifugation at 10,000 rpm for 45 min at 0°C. The supernatant, a transparent solution of protein (P1), was separated by decantation while the solid residue (P2) was washed with physiological saline and the pH adjusted to 8.6. The mean protein yield of subfraction P1 was 8 mg/g lung tissue, and of subfraction P2, 23 mg/g.

Altogether 30 antisera against the nucleoprotein fraction of the lungs of rats with experimental berylliosis, obtained by various schemes of immunization [3], were used. To remove antibodies

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TABLE 1. Relative Electrophoretic Mobility of New Antigens Appearing in Lungs in Experimental Berylliosis

Mobility	Antigen No.					
	I	II	III	IV	V	VI
Mean value of relative mobility	1,06	0,77	0,57	0,50	0,43	0,5—0,1
Zone	Albumin	α_1 Globulin	α Globulin	β Globulin	β_2 Globulin	γ Globulin

TABLE 2. Detection of Beryllium-Containing Antigens in Subfractions of Lung Nucleoproteins of Rats with Experimental Berylliosis by Active Anaphylaxis and by Zil'ber's Method

Expt. No.	Character of antigen			No. of animals	
	on sensitization	on desensitization	on reacting injection	total	with shock
1	P1	Not desensitized	Homologous serum + $BeCl_2$	10	6
	Not sensitized	» »	The same	4	0
2	P2	Not desensitized	Homologous serum + $BeCl_2$	7	6
3	Homologous serum + $BeCl_2$	Not desensitized	P2	10	4
	Homologous serum	» »	P2	4	0
4	H1+BeO	H1	P1	9	8
5	H2+BeO	H2	P2	8	7
6	P2	H2	H2+BeO	7	6
	Not sensitized	Not desensitized	H2+BeO	5	0
7	P2	(C+BeO) +(H2+BeO)	P2	8	7

against normal antigens, the resulting antiserum was exhausted by proteins of the nucleoprotein fraction of the lungs of intact rats. Completeness of exhaustion was verified by agar-diffusion tests. Antibodies were isolated together with the γ-globulin fraction by the Rivanol method or on DEAE-Sephadex [7] and concentrated with ammonium sulfate [6] by means of Sephadex G-25. The electrophoretic mobility of the individual antigens was calculated relative to the mobility of human serum albumin.

Comparison of the beryllium-containing proteins obtained in vitro with the lung antigens in experimental berylliosis was carried out on guinea pigs by active anaphylaxis and by Zil'ber's biological method. To prepare beryllium-containing antigens beryllium oxide dust was added to the subfractions of the "supernatant" from the lungs of intact rats (H1) and "residue" (H2) in the proportion of 100 μg per mg protein and mixed for 5 h daily for 1 week at room temperature on a magnetic mixer, the protein solution being kept for the rest of the time in a refrigerator. Guinea pig serum proteins to which beryllium chloride solution was added to the same concentration ex tempore was also used as artificial beryllium-containing antigen. The guinea pigs were sensitized subcutaneously with 2-4 mg of the corresponding protein solutions, and 20 days later a desensitization course and the reacting injection were given.

EXPERIMENTAL RESULTS

Immunoelectrophoretic investigation of the nucleoproteins from lungs with experimental berylliosis, using different antisera exhausted with antigens of normal lung tissues for development, revealed six antigens which were absent in the corresponding fraction of the lungs of intact rats. It must be pointed out that it was extremely difficult to obtain complete exhaustion of antibodies against lung tissue antigens. Despite

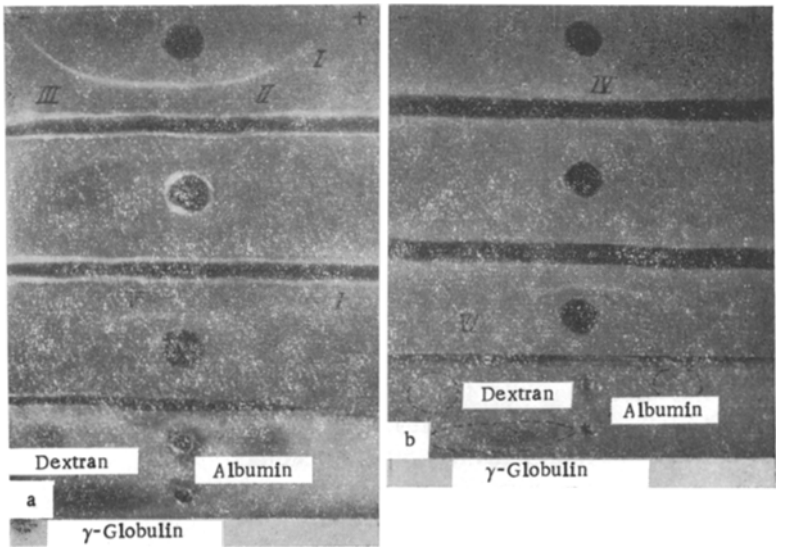

Fig. 2. Immunoelectrophoresis of nucleoproteins of lung fractions from rats with experimental berylliosis. Central wells contain nucleoproteins from lungs of intact rats, upper (a) and lower (b) wells contain subfraction of nucleoprotein fraction of lungs of rats with experimental berylliosis, readily soluble in physiological saline; lower (a) and upper (b) wells contain sparingly soluble subfraction of these proteins; gutters contain rabbit γ-globulins against nucleoprotein fraction of lungs of rats with experimental berylliosis (a – No. 21; b – No. 39) exhausted with proteins of nucleoprotein fraction from lungs of intact rats.

frequent repetition of the absorption of the antiserum, in most cases individual antigens reappeared after concentration, although they were readily distinguished by electrophoresis from the antigens of the pathologically changed lungs. A diagram of the arrangement of all these antigens, summarizing the results of several experiments, is given in Fig. 1. The results of individual immunoelectrophoretic investigations showing antigens absent from the normal lung are illustrated in Fig. 2. One of these antigens was found only in one subfraction; antigens II and VI were found only in subfraction P1, and antigen IV only in subfraction P2. The values of the relative electrophoretic mobility are given in Table 1.

A characteristic feature of most of the newly discovered antigens is the considerable extent of their precipitation bands. This was evidently because they consist of proteins which are serologically identical but which differ in their charge and molecular weight. Antigen VI, for example, gave two distinct arcs of immunoelectrophoresis which joined one another, so that two values of electrophoretic mobility, one for each arc, are given for this antigen in Table 1. Antigens with the mobility of α_2- and β-globulins were found most frequently. Special test systems were chosen for antigens IV, V, and VI. With their aid these antigens were found in most tissue extracts from the various organs of the experimental animals. However, the sensitive test system demonstrated the presence of these antigens in small quantities in certain tissues of the intact animals also. For instance, antigen VI was found also in the kidneys and it gave a reaction of partial identity with the lungs of intact rats, antigen V was found in heart tissue, and IV in the tissues of the stomach, liver, and lungs. Antigen IV evidently simply accumulates in the tissues in berylliosis; under normal conditions there is so little of it that it cannot be detected in a system with low sensitivity.

Since none of the antigens found was available in a purified form, the presence of beryllium in them was studied by the anaphylaxis method and by Zil'ber's biological method. By these methods it was possible to compare the additional antigens appearing in experimental berylliosis in vivo with antigens of beryllium-containing proteins prepared in vitro. The scheme and results of these experiments are given in Table 2.

The results of these experiments demonstrate the presence of cross-reacting antibodies against antigens formed in vivo and artificial beryllium-containing proteins. The conditions of experiments Nos. 1-6 were such that only beryllium could be the common determinant. Consequently, both subfractions of lung nucleoproteins of rats with experimental berylliosis may have beryllium-containing antigenic groups as components.

The results of these experiments thus demonstrate conclusively that several new antigens with high and low electrophoretic mobility appear in the lungs in berylliosis, and that some of them may possibly contain beryllium in their active group. In connection with these results it is imperative to recall observations [13] that the beryllium in the lungs migrates during electrophoresis both to the anode and to the cathode, which suggests that the metal can be bound with different tissue protein fractions.

However, additional antigens not containing beryllium also appear in the lungs in experimental berylliosis. This was shown by the results of experiment No. 7. In this experiment the animals were sensitized with proteins of subfraction P2. At the height of sensitization their blood serum was completely exhausted with antigens of normal lung tissue, with beryllium-containing proteins, and with antigens not specific for berylliosis but characteristic of the lungs during silicosis. Nevertheless, seven of the eight guinea pigs developed anaphylactic shock after the reacting injection of subfraction P2.

The nature of the new antigens discovered and the degree of their specificity for berylliosis are not yet known, and there is no direct evidence that they play an active role in the pathogeneis of the disease. Some of them are perhaps directly concerned with the production of autoimmune lesions, while others may be simply "markers" of immunopathological disturbances affecting many tissues in berylliosis.

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