DIFFERENCE BETWEEN RHEUMATOID FACTORS AND NORMAL IMMUNOGLOBULINS REVEALED BY THE MONOMOLECULAR LAYER TECHNIQUE

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The monomolecular layers technique has proved useful in the study of the stability of the structure and properties of the hydrophilic-lipophilic balance between immunoglobulins (Ig) of the G and M classes [3, 4]. With respect to some parameters it has proved possible to detect differences between normal Ig and Ig secreted in myelomatosis and Waldenström's disease [1, 5].

Rheumatoid factors (RF) contained in the blood serum of patients with rheumatoid arthritis, which are autoantibodies specifically recognizing determinants of the Fc-region of the IgG molecule, are also proteins of immunoglobulin nature [14]. Data have recently been published to show that RF differ in their structure from serum Ig of the same classes, which do not possess autoantibody activity. In recent studies [9, 10, 13] these changes have been linked with the carbohydrate part of the RF molecule. It has been shown that IgG-RF of patients with rheumatoid arthritis (RA) and with primary osteoarthritis (OA) differ from normal serum IgG in the level of galactosylation of their complex oligosaccharides, but they do not contain any new oligosaccharides by comparison with normal IgG [13]. From the chemical point of view RF is an Ig, which is hyposialized in the hinge region [10].

It was accordingly decided to investigate Ig with the properties of RF by the monomolecular layers method, in order to obtain additional information on whether they are analogous to normal serum IgG and IgM.

EXPERIMENTAL METHOD

Seropositive blood sera from 11 patients with RA were used. Chromatography was carried out on columns measuring 2 × 5 and 1 × 30 cm. As the affinity sorbent we used a new sorbent based on macroporous glass, treated with a copolymer of acrylic acid and its p-nitrophenyl ester derivative. Gel-filtration was carried out on Sephacryl S-300 (Pharmacia, Sweden) and Ultragel AcA-34 (France). QAE-Sephadex A 50 (Pharmacia) was used for ion-exchange chromatography. Immunosorbents were obtained by condensation of human IgG, isolated by ion-exchange chromatography of QAE-Sephadex A 50 in 0.005 M phosphate buffer, pH 7.3, from a 10% solution of γ-globulin for injection, with modified macroporous glass [15]. At each stage of isolation of proteins, the purity of the isolated preparations was verified immunochemically by Ouchterlony's double immunodiffusion test and by Grabar's technique of immunoelectrophoresis [11].

The RF preparations were isolated by two approaches, based on specific immune sorption of RF on immobilized human IgG: 1) isolation of RF from serum with isolation of free RF and incomplete immune complexes (IC) on an affinity sorbent with immobilized human IgG in the first stage, and subsequent gel-fitration on Ultragel AcA-34 in the second stage [7]; 2) isolation of RF proceeded by dissociation of IC directly in native serum as a result of acidification [14].

Surface pressure was measured by the glass plate balancing method [2]. The accuracy of the measurements was ±0.15 mN/m. Dependence of surface pressure π on area A was determined at various time intervals t after application of protein to the surface of the water in the bath by careful layering of a definite volume of a solution of RF of known concentration

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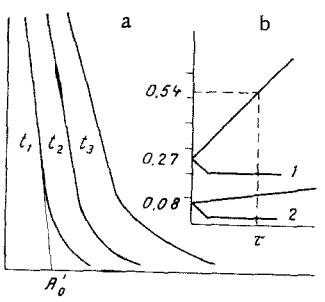

Fig. 1

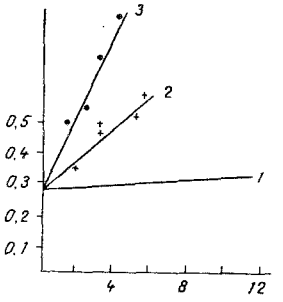

Fig. 2

Fig. 1. Determination of orientation of native IgG molecules and degree of their conformational stability (τ). a) Series of π—A curves and derivation of values of A_0 from them. Abscissa, area of monolayer (in m^2/mg); ordinate, surface pressure (in mN/m); b) typical A_0—t dependences and determination of A_0' and τ. Abscissa, time of formation of monolayer (in min); ordinate, area per molecule in monolayer (m^2/mg). 1) A_0 corresponds to horizontal orientation; 2) A_0 corresponds to vertical orientation.

Fig. 2. Dependences of limiting area A_0 on time of monolayer formation. 1) Normal IgG and IgM; 2) IgM-RF; 3) IgG-RF. A_0 for IgM calculated for the monomer. Abscissa, time of monolayer formation (in min); ordinate, area per molecule in monolayer (in m^2/mg).

from a micropipet. To discover whether the protein dissolves or not in the underlying solution, widely different (by 2-4 times) quantities of RF were used to form the monolayer. By coincidence of the π—A curves for differing values of t it was concluded that the monomolecular layer formed by the particular protein was stable.

Since RF was isolated by methods differing from the methods of isolation of normal serum Ig, it was necessary to isolate several control preparations and to test them by the monolayer technique in order to remove any suspicion that the difference in properties of RF was due to differences in the way of their preparation. These control preparations were serum Ig from patients with RA, which did not possess RF activity and preparations of normal IgG, treated by the method of isolation of RF.

EXPERIMENTAL RESULTS

The main characteristic of the substance obtained by the monolayer technique is the area which one molecule occupies on the phase boundary. With respect to proteins which are subject to surface denaturation, as a result of which the area of the molecule in the monolayer increases with time, the dimensions of the native molecule may be determined as follows. From the compression isotherm of the monolayer (the π—A curve), i.e., dependence of surface pressure developed by the monolayer during different degrees of compression, information is obtained about the area A_0 occupied by one protein molecule, at the given time t of existence of the monolayer (Fig. 1). On the basis of a series of π—A curves corresponding to different values of t, kinetic curves of A_0—t dependence are plotted and they intercept on the ordinate segments A_0 which indicate the area of the native protein molecule in the layer. It was shown previously [5] that only two values need to taken for IgG and IgM to calculate A_0 for the monomer, namely 19 and 66 nm^2 per molecule (0.08 and 0.27 m^2/kg), respectively. These values are close to those of the area of the least and greatest cross-sections of the IgG molecule obtained from the x-ray structural analysis data [8]. This is connected with the vertical or horizontal orientation of these molecules on the phase boundary respectively.

We know [3] that normal IgG and IgM, irrespective of their specific origin, are always oriented horizontally in monolayers on a surface of water-air type; their structure is very resistant to surface denaturation (this means that for a long time the monolayers consist of virtually native molecules); they form stable layers on substrates containing NaCl in concentrations of 0.15-0.6 M (Fig. 2).

TABLE 1. Orientation and Conformational Stability of RF Before and After Dialysis

No. of protein	Class of RF	Does RF form a stable monolayer?	A_0, m^2/mg	Orientation of RF in monolayer	τ, min	Characteristics of RF after dialysis
1	IgM	Yes	0,25	Horizontal	6,0	Normal
2	IgG	No	—	—	—	Solubility reduced
3	IgM	Yes	0,30	Horizontal	4,5	—
4	IgG	»	0,27	»	3,0	A_0=0,26, τ=8,5
5	IgG	»	0,29	»	3,0	Normal
6	IgM	»	0,27	»	65	—
7	IgG	No	—	—	—	—
8	IgM	Yes	0,27	Horizontal	81	—
9	IgG	»	0,26	»	8,0	—
10	IgM	No	—	—	—	—
11	IgG	Yes	—	—	<2,5	Normal
12	IgM	»	0,25	Horizontal	6,0	—
13	IgM	No	—	—	—	Unchanged
14	IgG	Yes	0,26	Horizontal	3,0	A_0=0,25, τ=13
15	IgM	»	—	—	<2,0	A_0=0,26, τ=8,5
16	IgG	Yes	0,24	Horizontal	4,5	—
17	IgM	»	—	—	<2,0	Unchanged
18	IgG	»	—	—	<2,0	The same
19	IgM	»	0,26	Horizontal	49	—
20	IgG	»	0,28	»	80	—
Normal	IgG	Yes	0,27	Horizontal	70	—
»	IgM	»	0,27	»	70	—

Legend. A_0 for IgM is calculated for the monomer.

It is clear on the basis of the above remarks that when the monolayer technique is used the following abnormalities can be detected for pathological Ig: 1) a vertical orientation; 2) instability of the native structure. As an example of such proteins we show in Fig. 2 straight lines of the A_0–t dependence for RF of two patients with RA. The extreme case of this pathology is that when the rate of loss of native structure by the molecules is so great that it is impossible to draw a reliable kinetic straight line and, consequently, it is impossible to determine the orientation of the molecules in the monolayer; 3) the hydrophilization of the surface of the protein globules, which may cause the Ig molecules to cease to form stable monolayers on salt solutions as a result of their dissolving the aqueous phase.

Altogether 20 proteins possessing the properties of RF were tested, and proteins of two classes at the same time – IgG and IgM – were isolated from nine patients. Data obtained from π–A curves for monolayers of these proteins formed on 0.5 M NaCl are given in Table 1. To characterize the conformational stability of the molecules, the value of τ is given in Table 1: it represents the time during which the area occupied by an Ig molecule on the phase boundary doubles itself compared with the area of the native molecule, which is 0.54 m^2/mg (Fig. 2).

Only three proteins closely resemble the normal in their behavior in monolayers (Nos. 6, 8, and 20). However, in all three cases the RF of another class was found to have pathological features. Abnormalities were found in relation to two parameters. In four cases increased hydrophilicity (Nos. 2, 7, 10, and 13), and in 13 cases a significant decrease in conformational stability were observed. The two types of molecular pathology can be attributed to the same cause if it is suggested that RF is a complex of the Ig molecule with another small molecule. For instance, we observed a similar change in the properties of the normal human IgG molecule when one or two molecules of Na deoxycholate were added to it [6]. In cases when the quantity of preparation isolated was sufficient, we therefore subjected it to dialysis. In the course of dialysis some of the proteins were changed. This change essentially was that the rapidly denaturing proteins become more resistant (the value of τ increased), and one of the pathologically soluble proteins became less soluble (No. 2), i.e., the change in properties of the proteins tended toward normal. Three proteins (Nos. 1, 5, and 11), according to the parameters tested, actually became indistinguishable from normal. This change in the properties of the proteins can be explained only on the grounds that dur-

ing dialysis certain small molecules, previously components of RF, passed into the dialysis fluid, and this led to normalization of these pathological Ig to some degree. It can naturally be assumed that in cases when no such changes took place (Nos. 13, 17, 18), RF nevertheless was a complex of two molecules, but with higher affinity for each other. Incidentally, on the basis of our data it cannot be concluded that the Ig which are components of RF are absolutely identical to normal Ig, for only three of the 10 proteins dialyzed became indistinguishable from normal. We consider that the difference between these Ig ought to exist, and it is manifested as abnormal affinity of Ig-RF toward certain small molecules. After addition of the latter the RF may perhaps become capable of forming complexes with the individual's own IgG.

It can thus be shown by the monolayers method that proteins possessing the properties of RF, isolated from the blood serum of patients with RA, behave differently from normal serum Ig. It follows from the results of the analysis that at least some, and possibly all, RF are Ig modified by small molecules. Carefully conducted control experiments will rule out the possibility of addition of these molecules during isolation of RF.

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MITOGENIC ACTION OF *Legionella pneumophila*: RATIO OF PROLIFERATING T AND B CELL CHANGES AFTER IMMUNIZATION

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Despite recent investigations by several groups of workers [7] the mechanisms of development of protective immunity in legionelloses have not been discovered. It is not clear what type of immune response (humoral or cellular) plays the leading role in the conditions of infection associated with legionellas. According to data in the literature, the serum (but not lymphocytes) of guinea pigs immunized with *L. pneumophila* antigens (LPA) can transfer protective immunity in the case of intratracheal infection [4]. On the other hand, there is evidence that after intraperitoneal immunization of guinea pigs with killed *L. pneumophila* cells the animals become resistant to intraperitoneal, but not to aerosol infection [5], although immunization leads to synthesis of antibodies against *L. pneumophilia* in high titers.

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