POLYANIONS INHIBIT BINDING OF AUTOANTIBODIES WITH SOME CELL PROTEINS

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In systemic lupus erythematosus (SLE), scleroderma, and other collagenoses, autoantibodies to different forms of DNA and RNA, to ribonucleoproteins, to proteins of the nucleus, ribosomes, cytoskeleton, and so on, are often found in the patients' serum [2, 10]. Crossed reactions with biopolymers carrying negatively charged groups have been described for many autoantibodies. For instance, autoantibodies to DNA react with phosphate groups of phospholipids [7], and with sulfate and carboxyl groups of proteoglycans [4, 5]. It is evident that reactions of this type take place on account of electrostatic interaction and they can be inhibited by polyanions, which compete with the antigen. Dextran sulfate, for example, binds with antibodies to DNA, preventing the formation of the immune complex [12]. However, the mechanism of interaction of autoantibodies with proteins has not yet been fully explained. Hardly anything is known about the effect of polyelectrolytes on binding of antibodies with proteins and on the contribution of electrostatics to immunoreactivity of protein antigens. In the present investigation the immunoblotting method was usid to examine the inhibitory effect of polyanions, which block the reaction of Ig from the serum of patients with SLE and scleroderma with certain cell proteins.

EXPERIMENTAL METHOD

Sera from patients with SLE (n = 108) and scleroderma (the "cross"-Academy of Medical Sciences of the USSR (Moscow). Serum from healthy blood donors (n = 20) and patients with lymphogranulomatosis (n = 17) were obtained at the Research Institute of Medical Radiology, Academy of Medical Sciences of the USSR (Obninsk). Monospecific "reference" sera to antigens Sm, U-RNP, La, Ro, and Scl-70 were obtained from the Slovak Institute of Rheumatology (Piešt'any, Czechoslovakia). Mouse monoclonal antibodies to cytoskeletal proteins (vimentin, vinculin, and tubulin), and also rabbit polyclonal antibodies (IgG fraction) to vinculin, myosin, and filamin, were generously provided by A. M. Belkin (All-Union Cardiologic Scientific Center, Academy of Medical Sciences of the USSR, Moscow).

Cultures of HeLa cells, human skin fibroblasts, and nonsecreting mouse myeloma were grown on DMEM medium containing 10% bovine serum, (10^8) were lysed in 5 ml of buffer containing 40 mM Tris, 10 mM EDTA, 4% SDS, 5% mercaptoethanol, 20% glycerol, and 2 mM PMSF (pH 8). The cell proteins were fractionated electrophoretically [8], using 3% concentrating and 5-15% gradient separating gel. Lysate from $0.5 \cdot 10^6$ cells was added to one well in the gel. After electrophoresis the proteins were transferred from the gel to a nitrocellulose filter [11] and the excess of adsorbing groups was blocked by 50 mM Tris-HCl buffer, pH 7.5, containing 0.5 M NaCl, 0.05 M Tween-20, 2% bovine serum albumin, and 0.3% gelatin. The prepared replicas were incubated for 2 h with sera and antibodies, diluted 30-50-fold with the blocking buffer described above. After washing with 50 mM Tris-HCl (pH 7.5) with 0.5 M NaCl and 0.5% Tween-20, the replicas were treated with antibodies against human Ig (Czechoslovakia), mouse Ig ("Miles"), or rabbit Ig ("Sigma") respectively, conjugated with peroxidase. Bands corresponding to antigens were developed with 0.05% 4-chloro-1-napthol with 0.03% H_2O_2. To determine the molecular weight of the antigens, standards from "Pharmacia" were used. Dextran sulfate 500,000 ("Ferak," Berlin), DNA and polyI ("Calbiochem"),

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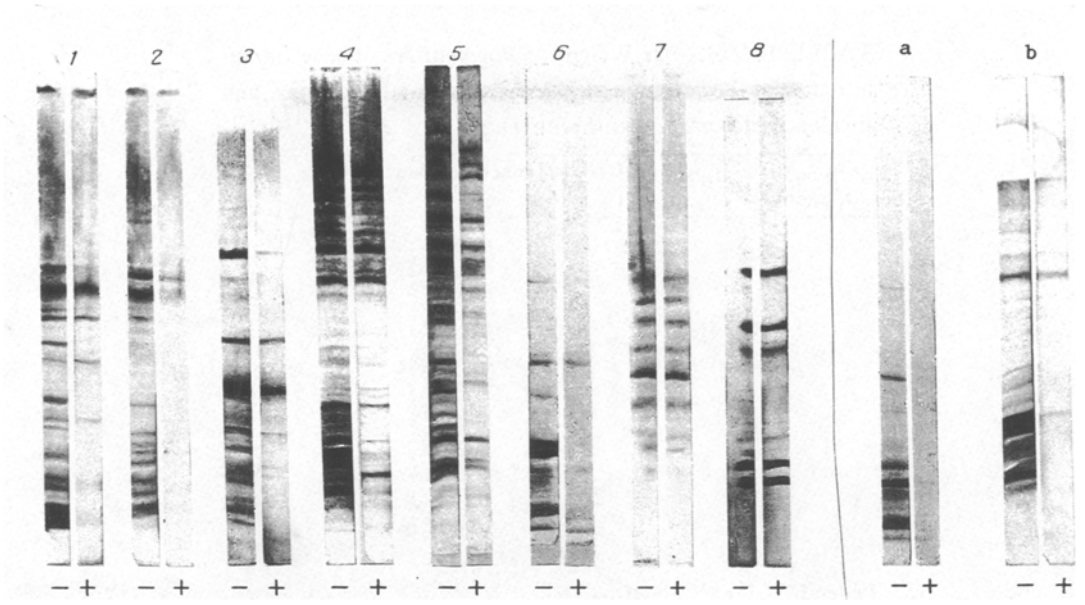

Fig. 1 Fig. 2

Fig. 1. Immunoblotting of extract of human skin fibroblasts. Horizontal lines on left indicate position of molecular weight standards (from top to bottom: 200, 94, 67, 43, 30, and 14 kD). 1, 2, 4) reaction with sera of patients with SLE, 3) of patient with scleroderma, 5) blot treated with serum of patient with lymphogranlomatosis, 6) serum of healthy blood donor, 7) negative control (blot treated only with conjugate with peroxidase), 8) reaction with monoclonal antibodies to vimentin (57 kD). Here and in Fig. 2, reaction without dextran sulfate, +) in presence of dextran sulfate (200 g/ml).

Fig. 2. Immunoblotting of extract of mouse myeloma cells: a) reaction with affinity-isolated autoantibodies to DNA, b) reaction with IgG, not bound with native DNA.

and heparin ("Fluka") were used in the inhibition reactions. The IgG fraction was isolated on a column with protein-A-sepharose. The IgM fraction was isolated by gel-filtration on sephacryl S-300. Autoantibodies to native DNA were obtained from IgG of patients with SLE by affinity chromatography on DNA-cellulose [9]. $F(ab)_2$ fragments were obtained by treating IgG with pepsin, immobilized on microcarriers, followed by centrifugation and passage of the supernatant through protein-A-sepharose.

EXPERIMENTAL RESULTS

Screening sera from patients with SLE and scleroderma by immunoblotting showed that dextran sulfate inhibits binding of autoantibodies with certain cell proteins. Of all the samples tested about 80% of sera (SLE and scleroderma) contained autoantibodies sensitive to the presence of dextran sulfate, which inhibited the reaction with one or several polypeptides of varied molecular weight (Fig. 1, 1-3). In about 20% of sera dextran sulfate did not affect autoantibody activity (Fig. 1,4). Besides dextran sulfate, other polyanions also had a similar inhibitory property: denatured DNA, polyI, and heparin (data not given). Polyanions were shown to give an inhibitory effect only in the case of their direct contact with autoantibodies, and preliminary treatment of the replicas with polyanions (before inhibition with the sera) did not lead to any decrease in Ig binding. Polyanions evidently block the reaction of autoantibodies with cationic binding centers, with negatively charged protein domains. No such effect was observed by testing sera from healthy blood donors and patients with lymphogranulomatosis (nonautoimmune disease) — these samples did not contain antibodies reacting with cell proteins (Fig. 1, 5-7). It was also shown that polyanions have no effect on activity of induced monoclonal and polyclonal antibodies to 1 vinculin, tubulin, myosin, or filamin (data not given) and vimentin (Fig. 1, 8). Investigation of the distribution of antigens reacting with autoantibodies showed that they are present in cells of different type and origin: polyanions inhibited the reaction of Ig with polypeptides of the same molecular weight in extracts of HeLa cells, human skin fibroblasts, and mouse myeloma cells. It was shown with the aid of monospecific sera that these polypeptides are not components of Sm, U_1-RNP, La, Ro, and Scl-70 antigens, and they can evidently be isolated into a special subclass of autoantigens, whose immunoreactivity is sensitive to the presence of polyanions. Values of molecular

TABLE 1. Molecular Weight of Polypeptides Whose Immunoreactivity Is Sensitive to the Presence of Polyanions, and Incidence of Sera Reacting with Them

Molecular weight of antigen, kD	Incidence of positive sera, %
18	15 (SLE)
22	26 (SLE)
26	15 (SLE)
30	30 (SLE)
33	40 (SLE)
37	21 (SLE), 30 (SCD)
42	20 (SLE)
46	26 (SLE)
51	9 (SLE)
58	11 (SLE)
82	7 (SLE)
96	3 (SLE)
108	11 (SLE)
120	25 (SLE)
150	14 (SLE)
200	5 (SLE)

Legend. SCD) scleroderma.

weights of the most reactive ("major") antigens from this subclass and the frequency of occurrence of sera reacting with them are given in Table 1.

Analysis of the Ig fractions showed that the inhibitory effect of the polyanions was manifested both for IgG and for IgM. Activity of the $F(ab)_2$ fragments obtained from IgG of patients with SLE and scleroderma also was inhibited by dextran sulfate; this fact is evidence that polyanions inhibit the specific antigen-antibody reaction, and that the effect we observed was unconnected with interaction of antigens with the *Fc*-fragment or with nonimmunoglobulin components of immune complexes present in the serum. The study of IgG subfractions from patients with SLE showed that dextran sulfate inhibits the reaction both of affinity-isolated antibodies to native DNA and of IgG unbound with the affinity sorbent (Fig. 2), with proteins. Cross reactions with proteins [1, 3] and also reactions which may probably be inhibited by polyanions with greater affinity for antibodies than protein molecules, have been described for autoantibodies to DNA. It is a surprising fact that polyanions block binding with proteins of those IgG which do not react with native DNA (Fig. 2b). The possible explanation is that these antibodies can react only with single-chain polysulfates and polyphosphates, and the reaction with native DNA is hindered by steric factors connected with the arrangement of the phosphate groups on the double helix.

Thus polyanions inhibit binding of autoantibodies frequently found in the sera of patients with SLE and scleroderma, with certain proteins. Reactions of this kind are probably based on electrostatic interaction between oppositely charged sites on molecules of the antigen or polyanion and Ig. This interaction, despite its relative specificity, leads to the formation of quite firm complexes, resistant to a high ionic strength (0.5 M NaCl). These autoantibodies can evidently play an important role in the pathogenesis of autoimmune diseases, reicting with different anionic structures of biomolecules. For example, their binding with negatively charged glycosaminoglycans, which are components of the glomerular membrane and intercellular matrix [6], will facilitate destruction of connective tissue, the development of nephritis and vasculitis, and skin lesions, which are often observed in SLE and scleroderma.

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SUPPRESSION OF THE IMMUNE RESPONSE BY LUNG CELLS IN EXPERIMENTAL TUBERCULOSIS

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Infection of mice by virulent *Mycobacterium tuberculosis* cells is accompanied by generalized infection with bacteriemia, lymphadenopathy, and splenomegaly. The most marked pathological process develops in the lungs, which in tuberculosis are the site of direct interaction between pathogen and effector mechanisms of specific and nonspecific resistance [1].

Data which have now accumulated are evidence that lung tissue contains all the principal types of immunocompetent cells, including T and B lymphocytes, macrophages, dendritic cells, and natural killer cells [3, 5, 6], but their particular features in tuberculosis are unknown. This paper gives the results of a study of the immunologic properties of the interstitial cells of the lung in mice with experimental tuberculosis, evidence of activation, as a result of infection, both of T lymphocytes specific for mycobacterial antigens and of suppressor cells, adherent to plastic, which suppress proliferation of immune T cells.

EXPERIMENTAL METHOD

Mice of the inbred CBA/Sto line aged 3-4 months were obtained from the "Stolbovaya" Nursery, Academy of Medical Sciences of the USSR. The mice were infected intravenously with a virulent strain of *M. tuberculosis* H37Rv in a dose of 25 μg/mouse. The mice were used in the experiments 3-7 weeks after infection. Sterile suspensions of the interstitial cells of the lung were obtained by enzymic dissociation. For this purpose the vascular bed was perfused with an intravenous infusion of 7 ml of Hanks' solution with antibiotics, containing 10 U/ml of heparin, after which bronchoalveolar lavage was carried out with warm physiological saline containing antibiotics, 1-1.5 ml of solution being injected through a cannula inserted into the trachea, followed by aspiration of the contents, the procedure being repeated eight times. The lungs were removed from the chest, perfused another twice in medium 199 containing antibiotics, cut into small pieces measuring about 1-2 mm^3, and added to a solution containing 2 mg/ml of type I collagenase (260 IU/mg, from "Boehringer," West Germany) in medium L-15 containing 20 mM HEPES, 1% embryonic calf serum (ECS), 50 U/ml of kanamycin (all components from "Flow Laboratories," England), and 50 μg/ml of DNase (USSR). The sample was incubated at 37°C for 90 min on a planchet shaker (ABP-1).

A unicellular suspension was obtained by repeated pipetting of the suspension and passing it through a metal sieve. The cells were then washed 3 times by centrifugation at 150 g for 10 min and filtered through a cotton wool filter. The suspension thus obtained consisted of single cells with a viability of 80-90%. To remove cells adherent to plastic the suspension was incubated in complete nutrient medium (RPMI-1640, containing 10% ECS, 10 mM HEPES, 4 mM glutamine, 50 U/ml kanamycin, 1% of nonessential amino acids, 2.2 mM pyruvate, and $5 \cdot 10^{-5}$ 2-mercaptoethanol; all components from "Flow Laboratories," England), on plastic Petri dishes 90 mm in diameter for 2 h at 37°C in a CO_2-incubator in a dose of $(25\text{-}30) \cdot 10^6$ cells per dish. Adherent cells were removed mechanically after incubation of the monolayer with a cold solution containing 0.02% EDTA for 30 min at room temperature.

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