LITERATURE CITED

1. B. D. Brondz, S. G. Egorova, and I. F. Kotomina, Eur. J. Immunol., 5, 733 (1975).
2. B. D. Brondz, A. V. Karaulov, and I. F. Abronina, Mol. Biol., 13, 1287 (1979).
3. B. D. Brondz, A. V. Karaulov, I. F. Abronina, et al., Genetika, No. 12, 2119 (1979).
4. B. D. Brondz, A. V. Karaulov, and I. F. Abronina, Tsitologiya, No. 5, 583 (1980).
5. B. D. Brondz, A. V. Karaulov, and Z. K. Blandova, Immunologiya, No. 1, 43 (1980).
6. B. D. Brondz and O. V. Rokhlin, Molecular and Cellular Bases of Immunologic Recognition [in Russian], Moscow (1978).
7. B. D. Brondz, E. Ya. Khachikyan, G. I. Drizlikh, et al., Byull. Éksp. Biol. Med., No. 6, 723 (1977).
8. A. Eisenthal, D. Nachtigal, and M. Feldman, Cell. Immunol., 34, 112 (1977).
9. K. M. Ferguson and R. L. Simmons, Transplantation, 25, 36 (1978).
10. F. W. Fitch, U. D. Engers, J. C. Cerottini, et al., J. Immunol., 116, 716 (1976).
11. J. L. Glick, C. Lockwood, J. Williams, et al., Transplantation, 18, 86 (1974).
12. T. Hirano and A. A. Nordin, J. Immunol., 117, 2226 (1976).
13. R. J. Hodes and K. S. Hathcock, J. Immunol., 116, 167 (1976).
14. C. G. Orozs and F. H. Bach, J. Immunol., 123, 1419 (1979).
15. M. Röllinghoff, A. Starzinsky-Powitz, K. Pfizenmaier, et al., J. Exp. Med., 145, 455 (1977).
16. E. Sabbadini, J. Exp. Med., 140, 470 (1974).

INTERACTION OF THE C1q COMPONENT OF COMPLEMENT AND COLLAGEN WITH NUCLEIC ACIDS AND POLYANIONS

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The appearance of proteins which interact with nucleic acids (NA) in solutions with low ionic strength in the blood serum of patients with chronic inflammatory conditions was reported previously [3]. One such protein could be serum collagen-like glycoprotein C1q, reacting with NA [4, 6, 7, 11]. Like collagen, C1q is synthesized by fibroblasts and has a molecular weight of 400,000-500,000; its collagen-like region consists of 78 proline-hydroxyproline (hydroxylysine)-glycine triplets [5, 12, 13]. Interaction between C1q and the antigen–antibody complex or with a polyanion activates the I component of complement, including factors C1r and C1s [8].

The writers showed previously that collagen can interact with NA at neutral pH, and the hypothesis was put forward that these complexes play a role in the pathogenesis of diffuse connective tissue diseases and, in particular, of systemic lupus erythematosus [2].

In the present investigation interaction of the C1q component of complement and acid-soluble collagen with NA, and also the structures of NA which take part in interaction with these proteins, were studied.

EXPERIMENTAL METHOD

C1q was isolated from human blood [14] and collagen from rat skin [1]. The protein concentration was determined by Lowry's micromethod [9]. Labeled DNA was obtained by Marmur's method [10] with additional treatment with pronase from Escherichia coli strain W3110 cells, grown in the presence of ^{3}H-thymidine. The specific activity of the DNA preparations was 25,000-100,000 cpm/μg. Poly I and poly C were from Calbiochem, USA, polyU and polyA from Reanal, Hungary, Dextran sulfate (DS) from Ferak, Berlin; heparin was dissolved

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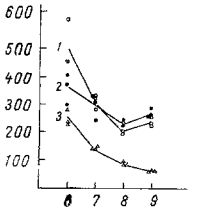

Fig. 1. Effect of pH and ionic strength of solution on interaction between C1q component of complement and ^{3}H-nDNA. 1) 0.05 M NaCl, 2) 0.1 M NaCl, 3) 0.15 M NaCl. Abscissa, pH; ordinate, radioactivity (in cpm) of ^{3}H-nDNA–C1q complex adsorbed on nitrocellulose filter.

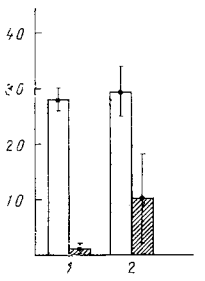

Fig. 2

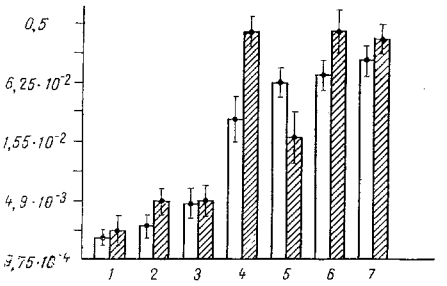

Fig. 3

Fig. 2. Effect of denaturation of C1q and of acid-soluble collagen on interaction with ^{3}H-nDNA. 1) C1q, 2) Acid-soluble collagen; unshaded columns denote native protein; shaded columns denatured protein. Ordinate, % of binding of ^{3}H-nDNA.

Fig. 3. 50% inhibition of interaction between C1q component of complement or acid-soluble collagen and ^{3}H-nDNA by polynucleotides and polyanions. Unshaded columns – C1q, shaded columns – collagen; 1) DS, 2) polyI, 3) poly U, 4) heparin, 5) polyC, 6) polyA, 7) polyI–polyC. Ordinate, quantity of inhibitor (in μg).

in buffer containing 0.05 M NaCl, 0.015 M Na citrate, and 0.005 M Tris-HCl, pH 8.0; RSC* = 0.085. The polyI–polyC hybrid was obtained by heating a mixture of equimolar amounts of polyI and polyC on a water bath at 70°C for 30 min, followed by slow cooling.

The effect of pH and ionic strength on interaction between C1q and ^{3}H-nDNA was studied by carrying out the reaction between them in buffer solution containing 0.05-0.1-0.15 M NaCl and 0.0015 M Na citrate, to each of which Tris-HCl buffer, pH 6.0-7.0-8.0-9.0 was added to a final concentration of 0.005 M. The protein content in the sample was that which bound approximately 50% of ^{3}H-nDNA (1500 cpm) added to a 0.05 M solution of NaCl, pH 8.0. After successive addition of 100 μl of the solution of C1q in 0.1 M NaCl, 0.015 M Na citrate, and 100 μl of the solution of ^{3}H-nDNA in the corresponding buffer the volume of the sample was made up with buffer to 900 μl. The sample was applied to a Synpore No. 8 nitrocellulose filter (Czechoslovakia) with a pore diameter of 0.25 μ.

Interaction between C1q, acid-soluble collagen, and NA was studied in the inhibition test in which they reacted with ^{3}H-nDNA, in buffer containing 0.05 M NaCl, 0.015 M Na citrate, 0.005 M Tris-HCl, pH 8.0. Solutions of inhibitor, C1q in buffer, or collagen in 0.01 N CH_3COOH, binding 50% of the subsequently added ^{3}H-nDNA, were added consecutively in volumes of 100 μl each (the final pH of the solution in this case was 7.4).

*Relative salt concentration – electrical conductivity corresponding to the electrical conductivity of a molar solution of NaCl at 0°C.

The volume of the sample was made up to 500 μl with buffer. The mixture was incubated for 30 min at 37°C (for C1q) or at 22°C (for collagen), then for 60 min at 4°C. The ^{3}H-nDNA–protein complex was adsorbed on a Synpore No. 8 nitrocellulose filter. Radioactivity was measured on a liquid scintillation counter (Intertechnique, France), in toluene scintillator consisting of 100 mg POPOP, 4 g PPO, 850 ml toluene, and 50 ml absolute ethanol. The result was expressed in percentage of binding of ^{3}H-nDNA or percentage inhibition of interaction of C1q or collagen and ^{3}H-nDNA.

EXPERIMENTAL RESULTS

Data on the effect of pH and ionic strength on interaction between C1q and ^{3}H-nDNA are given in Fig. 1. A fall of pH intensified this interaction whereas an increase in the ionic strength of the solution from 0.05 to 0.15 M NaCl inhibited the reaction. Denaturation of C1q and acid-soluble collagen, caused by heating them to 56°C for 30 min [2, 24], led to complete cessation of interaction between C1q and ^{3}H-nDNA, in agreement with observations by many workers on its inactivation [4, 14], and only partially reduced interaction of collagen with it (Fig. 2). These results are evidence that among proteins which appear in the blood serum of patients with chronic inflammatory conditions, and which interact with NA in solutions with low ionic strength, there are no appreciable quantities of C1q, for heating these sera to 56°C for 30 min did not reduce their ability to interact with Na; however, the possibility cannot be ruled out that collagen breakdown products may be among them [3].

The structures of NA with which the C1q component of complement and acid-soluble collagen interact were studied in the inhibition test during interaction between ^{3}H-nDNA and the above-mentioned protein, using the following synthetic polynucleotides and polyanions; polyI, polyC, polyU, polyA, polyI–polyC, DS, and heparin. Whereas heparin, polyI, polyU, and DS strongly inhibited interaction between C1q and ^{3}H-nDNA, its inhibition to the same degree required about 30 times more polyA, polyC, and the polyI–polyC complex. Similar results also were obtained during inhibition of interaction between acid-soluble collagen and ^{3}H-nDNA (Fig. 3).

Strong interaction of proteins with DS, polyI, polyU, and heparin indicates that it is determined by the polyanionic properties of heparin. The appearance of an exocyclic amino group in the structure of the nitrogeneous bases from which the polynucleotides are built inhibits their interaction with proteins even if this group lies within the double helix, which happens during the formation of the hybrid polyI–polyC molecule. Under these circumstances C1q and the acid-soluble collagen interact practically equally with purine and pyrimidine polynucleotides. These data are evidence of the important role of the collagen-like region of the C1q component of complement, which determines its interaction with NA.

LITERATURE CITED

1. V. N. Orekhovich, A. L. Tustanovskii, and N. E. Plotnikova, Dokl. Akad. Nauk SSSR, 60, 837 (1948).
2. A. M. Poverennyi, Biokhimiya, 24, 777 (1959).
3. A. M. Poverennyi, L. M. Tsyplyakovskaya, V. V. Boichuk, et al., Ter. Arkh., No. 9, 123 (1976).
4. L. A. Aarden, F. Lakmaker and T. E. Feltkamp, J. Immunol. Meth., 10, 26 (1976).
5. M. S. Al-Adnani and J. O'D. McGee, Nature, 263, 145 (1976).
6. V. Agnello, R. I. Carrn, D. Koffler, et al., Fed. Proc., 28, 696 (1969).
7. T. Borsos, H. J. Rapp, and C. Crisler, J. Immunol., 94, 662 (1965).
8. M. Loos and D. Bitter-Suermann, Immunology, 31, 931 (1976).
9. O. H. Lowry, N. J. Rosebrough, A. L. Farr, et al., J. Biol. Chem., 193, 265 (1951).
10. J. Marmur and P. Doty, J. Mol. Biol., 3, 585 (1961).
11. E. Raepple, H. U. Hill, and M. Loos, Immunochemistry, 13, 251 (1976).
12. K. B. N. Reid, Biochem. J., 141, 189 (1974).
13. K. B. M. Reid, D. M. Lowe and R. R. Porter, Biochem. J., 130, 749 (1972).
14. J. E. Volankis and R. M. Stroud, J. Immunol. Meth., 3, 25 (1973).