indomethacin, in a concentration of 1 μg/ml, abolished suppression of proliferation of lymphocytes from bronchial asthma patients, induced by PAF. Thus the effect of suppression by PAF may be connected with the fact that it induces stimulation of prostaglandin biosynthesis in monocytes; prostaglandins, especially those of type E, moreover, exhibit a marked suppressive effect on lymphocyte proliferation. However, the mechanisms of suppression of lymphocyte proliferation by PAF are evidently more varied and complex, for it has recently been shown that PAF suppresses the formation of interleukin-2, which is essential for PHA-stimulated lymphocyte proliferation, by lymphocytes [7]. The results of the present investigation and data in the literature indicate that PAF can be regarded as a new lipid bioregulator of cells of the immune system.

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SELECTIVE EFFECT OF CHORIONIC GONADOTROPHIN ON LYMPHOCYTE SUBPOPULATIONS

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Research workers are currently concentrating their efforts on the search for substances capable of regulating at will the immune response by selective action on individual stages of immunogenesis or on individual subpopulations of immunocompetent cells. The study of the immunomodulating ability of chorionic gonadotrophin (CG) is interesting from this point of view. The present writers [1, 3] and others [6-11] showed previously that CG has a dose-dependent inhibitory action in vitro and in vivo on cell-mediated immune responses. Data on the effect of CG on individual subpopulations of immunocompetent cells are not to be found in the literature.

The aim of the investigation described below was accordingly to study the effect of CG on different lymphocyte subpopulations: natural killer (NK) cells, suppressor T cells, effector T_{dth} cells.

EXPERIMENTAL METHOD

Pharacopoeial CG of Soviet origin and purified CG with biological activity of 30,000 IU/mg, obtained from the Moscow Endocrine Factory, were used. The doses used were comparable with physiological concentrations of CG determined in the blood in the early stages of pregnancy. There were four series of experiments in which 300 CBA and C57BL/6 mice were used. The effect of CG on induction and formation of specific suppressor T cells was studied by the method in

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TABLE 1. Effect of CG on Formation of DTH to SRBC in Mice (M ± m)

Scheme of injection of CG	Group of animals	Total number of animals	Weight of paw, mg	p
Control I (intact mice + reacting dose of SRBC)	1	15	25,0±2,3	—
Control II (immunized mice + reacting dose of SRBC	2	15	45,0±3,6	$p_{1-2}<0,01$
CG in a dose of 200 IU per mouse on day of immunization	3	15	24,0±1,6	$p_{2-3}<0,01$
CG in a dose of 200 IU per mouse on 5th day after immunization	4	15	28,0±2,7	$p_{2-4}<0,01$
CG in a dose of 200 IU per mouse (intact mice) + reacting dose of SRBC	5	8	27,0±1,7	$p_{1-5}>0,05$

TABLE 2. Effect of CG on NK Cell Activity in Vitro (M ± m)

Group of experiment	NK activity in absence of CG, %	NK activity in presence of CG, %		
		200 IU per mouse	500 IU per mouse	1000 IU per mouse
Control I	31,0±2,1	29,0±3,5	31,0±3,4	32,0±1,8
Control II	96,0±3,0	95,0±3,0	96,0±4,0	95,0±4,0
Experiment (K-562 cells + effector lymphocytes	50,0±2,5	48,0±2,1	43,0±4,8	30,0±3,7

Legend. Control I) spontaneous lysis of target cells, control II) index of viability of lymphocytes.

[14]. Spleen cells (5×10^7 cells) of CBA mice, 14 days after hyperimmunization with sheep's red blood dells (SRBC; 3×10^9 cells) were transplanted into intact mice simultaneously with an immunizing dose of SRBC. Functional activity of the suppressor cells was determined by the local hemolysis test [12] on the 4th day after transplantation of the cells. The CG was injected in a dose of 150 IU per mouse intravenously, 1, 3, and 7 days after hyperimmunization with SRBC (induction of suppressor T cells, experiments of series I) as a single injection during transfer of the cells into syngeneic recipients (mature suppressor T cells, experiments of series II), and also into immunized recipients without transplantation of donors' splenocytes (control for injection of CG).

In the experiments of series III to test delayed-type hypersensitivity (DTH) to SRBC, mice were immunized subcutaneously in the dorsal region (10^8 SRBC and 0.5 ml of 0.14 M NaCl; control I and II respectively). The reacting dose of SRBC (10^8 cells) was injected 5 days later into the left hind footpad, the right serving as the control (injection of 0.14 M NaCl only). The mice were killed 24 h after injection of the reacting dose (on the 6th day after immunization) and DTH was estimated as the difference in weight of the experimental and control paws. CG was injected in accordance with the following schemes: 1) on the day of immunization; 2) on the 5th day after immunization, to assess the effect of CG on precursors (scheme I) and mature effector T_{dth} cells, mediating the DTH reaction (scheme II) [13], and 3) into unimmunized mice (control for injection of CG).

In the experiments of series IV the effect of CG was studied on activity of NK cells, depending on dose [5]. Cells of the transplantable myeloid K-562 strain, labeled with ^{3}H-uridine, were used as target cells. To assess NK activity, the following parameters were determined: 1) the effect of CG on the target cells (control for injection of CG), 2) the viability of the NK cells in the presence of CG, and 3) NK activity on target cells under the influence of CG. The results obtained in all the experiments were subjected to statistical analysis by the Student—Fisher method.

EXPERIMENTAL RESULTS

CBA mouse spleen cells, 2 days after immunization with SRBC, on transplantation into syngeneic mice suppressed the development of the immune response. Three times fewer antibody-forming cells (AFC) were formed in the spleen of mice receiving splenocytes from immune donors and SRBC than in the control groups. This inhibition of the immune response was mediated by T cells and antigen-specific in character: the immune response was suppressed only to the antigen with which the donor of the spleen cells was immunized [4].

In the experiments of series I the effect of CG was studied on induction of specific suppressor T cells. The preparation was injected 3 times together with a suppression-inducing dose of SRBC 1, 3, and 7 days after immunization. During transfer of immune spleen cells,

treated beforehand with CG, into syngeneic recipients statistically significant suppression of the immune response ($p < 0.01$) was observed; the number of AFC was 7 times less than in the control groups and 2.5 times less than in the group of mice receiving an injection of suppressor T cells. It can be concluded from the results that CG has a stimulating action on induction of specific suppressor T cells. Injection of CG into recipient mice immunized with SRBC, without transfer of immune donors' splenocytes (suppressor T cells) led to a similar decrease in the number of AFC in the spleen (by 7-8 times), but starting only after a dose of 375 IU per mouse [2].

In the experiments of series II the effect of CG was studied on mature specific suppressor T cells. Together with immune spleen cells, the recipients were given a single injection of CG in a dose of 150 IU per mouse. The number of AFC was virtually the same as in the group of mice receiving suppressor T cells, i.e., CG had no inhibitory action on mature specific suppressor T cells. Thus the results of the two series of experiments are evidence that CG stimulates induction of specific suppressor T cells but does not affect the activity of mature suppressor T cells.

In the experiments of series III the action of CG was studied on the formation of DTH to SRBC. As Table 1 shows, the weight of the paws of the immunized mice after injection of the reacting dose of antigen (control II) was twice the weight of the paws of the intact animals (control I). After injection of CG in a dose of 200 IU per mouse on the day of immunization (scheme I), just as on the 5th day after immunization with SRBC (scheme II) the weight of the paws of the experimental animals was reduced approximately by half, and it corresponded to the weight of the paws of the control mice (control I). The results are evidence of an inhibitory action of the preparation both on precursor cells and on mature effector T_{dth} cells, mediating the DTH response to SRBC. These parameters in intact animals after injection of CG did not differ from the control.

In the experiments of series IV the effect of CG in doses of 200, 500, and 1000 IU per mouse on natural killer cell activity was studied (Table 2). The lytic activity of NK cells in cultures not treated with CG was 50%. The preparation had no action on the target cells, nor on the viability of the lymphocytes (controls I and II). In a concentration of 200 IU/ml CG did not affect the level of killer-cell activity (Table 2). With a dose of CG of 500 IU/ml lytic activity of the NK cells was 43%, compared with 30% after a dose of 1000 IU/ml. Only with a concentration of 1000 IU/ml was a decrease observed ($p < 0.05$) in lytic activity of the killer cells, i.e., the effect of CG depended on the dose of the preparation. The results are in agreement with data on reduction of NK activity of the blood plasma in pregnant women [9].

The investigations thus showed that CG, depending on the dose and time of its administration relative to the antigen, has a stimulating action on precursors of suppressor T cells, does not affect mature specific suppressor T cells, and has an inhibitory action on effector T_{dth} cells and NK cells, so that it can be classed as an immunomodulating agent. The ability of CG to act selectively on individual lymphocyte subpopulations provides a basis for its inclusion in the schedule of combined immunosuppressive therapy, which is being successfully used [2] in conjunction with allografting of endocrine organs.

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MONOCLONAL ANTIBODIES CROSS-REACTING WITH FIBROBLASTS OF MYOCARDIAL INTERSTITIAL CONNECTIVE TISSUE AND WITH GROUP A STREPTOCOCCAL CELL WALL PROTEIN ANTIGENS

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A cross-reacting antigen (CRA) was found previously among the nontype-specific antigens (NTSA) of the cell wall of the group A streptococcus [5]. Sera of rabbits immunized with NTSA, when tested by the indirect immunofluorescence method (IIFM), have been shown to react with myocardial interstitial connective tissue (ICT) cells and with cultures of fibroblasts [5]. Deposits of bound immunoglobulins have been discovered in the myocardial ICT of immunized animals. In spite of this, only heterophilic antibodies, reacting with ICT of other species of mammals, and not autoantibodies, have been found in the sera of these animals. The reasons why bound immunoglobulins are found in the myocardial ICT in spite of the absence of autoantibodies in the sera is not yet understood. The solution to this problem is interesting because a similar phenomenon, namely the discovery of heterophilic antibodies, and not of autoantibodies, in sera and of bound immunoglobulins in the myocardial ICT, has been described in rheumatic fever [6, 9].

The aim of this investigation was to obtain monoclonal antibodies (McAb) to cell wall NTSA of the group A streptococcus, cross-reacting with myocardial ICT cells and with fibroblasts of other mammalian organs, and also to examine the question of whether these antibodies are autoantibodies or not.

EXPERIMENTAL METHOD

BALB/c mice were immunized with a fraction containing a streptococcal cell wall NTSA. Cultures of group A streptococcus with enhanced virulence were used (type 5M 20/59 and type 29M 15/55, Prague Collection, obtained from Dr. J. Rotta, Czechoslovakia). The cultures were grown on medium with casein hydrolysate without addition of serum and were washed off 3 times with 0.85% NaCl solution, pH 7.2. HCl extracts were prepared by Lancefield's method. The NTSA fraction was isolated from the HCl extracts by preparative electrophoresis [10]. The fraction obtained from type 5 streptococci was injected intraperitoneally in a dose of 20 μg 8 times at intervals of 7 days. The last injection was given 3-4 days before removal of the spleen. The first dose of the fraction was injected together with Freund's complete adjuvant (FCA). Hybridomas and McAb were obtained by the usual method [12] as described previously [1]. On fusion of the splenocytes with a myeloma of strain SP-2/0, 50% polyethylene-glycol (mol. wt. 4000, "Serva") was used. To prepare the ascites fluids, 10 days after injection of 0.5 ml of pristane ("Sigma") the mice were given an intraperitoneal injection of 10^7 hybridoma cells. Supernatants were screened and McAb studied by the ELISA method as described previously [4]. Peroxidase-labeled rabbit antibodies to mouse immunoglobulins were used [the antibodies were obtained from the Laboratory of Immunologic Diagnosis (Director K. D. Shakhanina), N. F. Gamaleya Research Institute of Epidemiology and Microbiology, Academy of Medical Sciences of the USSR].

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