SPECIFIC REACTIVITY OF CYCLOPHOSPHAMIDE-INDUCED SEMIALLOGENEIC CHIMERAS

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After injection of cyclophosphamide (CP) and (CBA × C57BL/6)F_1 lymphoid cells into adult CBA mice long-term partial chimerism of the lymphoid tissue and tolerance to heterotopic transplantation of heart and skin of donor's origin are observed [3, 4, 7]. The presence of active mechanisms of tolerance was discovered in such chimeras by the writers previously: a serum blocking factor [2] and suppressor cells [6].

The aim of this investigation was to study the ability of T lymphocytes of semiallogeneic chimeras to carry out specific immune recognition in mixed lymphocyte culture (MLC) reactions and a local graft versus host reaction (GVHR) and also to determine the immunoreactivity of the chimeras in the delayed-type hypersensitivity (DHT) test and by the skin grafting method at different times after creation of chimerism.

EXPERIMENTAL METHOD

The experimental animals were male CBA, C57BL/6, and BALB/c mice and also male (CBA × C57BL/6)F_1 hybrids weighing 18-20 g, obtained from the Stolbovaya Nursery, Academy of Medical Sciences of the USSR.

Semiallogeneic chimeras were obtained by injecting CP in a dose of 200 mg/kg intraperitoneally into adult CBA mice, and 3-6 h later, injecting 10^8 spleen cells from (CBA × C57BL/6)F_1 hybrid mice intravenously [4]. The control consisted of CBA mice receiving an intraperitoneal injection of CP alone (CBA_{cp}).

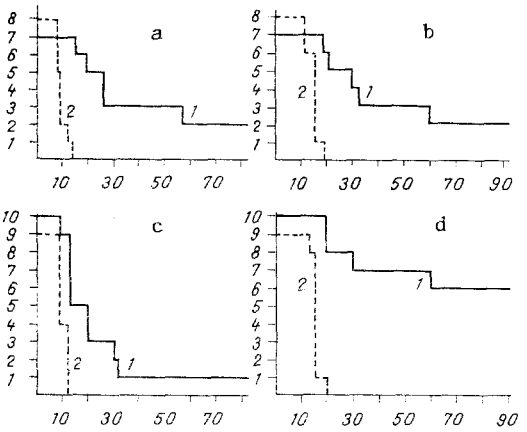

Fig. 1. Comparative survival time of skin grafts from C57BL/6 (1) and BALB/c (2) lines in semiallogeneic chimeras. Abscissa, time after transplantation (in days); ordinate, number of viable grafts. a, b) Beginning and end, respectively, of rejection after skin grafting 2.5 months after induction of tolerance; c, d) the same, when skin was grafted 4.5 months after induction of tolerance.

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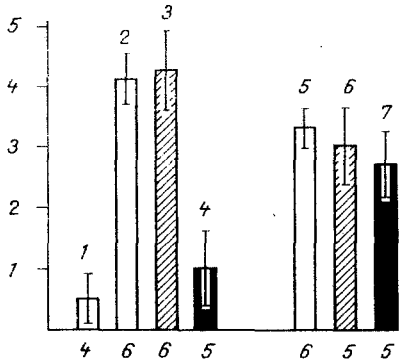

Fig. 2. Specific reduction of intensity of DTH reaction to C57BL/6 antigens in chimeras. Horizontal axis: top row of numbers denotes group of animals, bottom row — number of mice; vertical axis — size of focus of edema of paw (in units) after reacting injection of antigen into control intact CBA mice (unshaded columns), in chimeras (black columns), and in mice receiving an injection of CP (obliquely shaded columns); 2-4) animals immunized beforehand with C57BL/6 antigens; 5-7) immunization with BALB/c antigens.

Skin grafting was carried out by Billingham's method in Medvedev's modification [1]. A skin fragment was transplanted from the donor's tail to the recipient's back. The beginning of rejection (slight reddening, scaling, mild rigidity of the graft) and total rejection (necrosis) were recorded.

The DTH test was carried out by the method developed by Chernyakhovskaya et al. [9]. The experimental mice were immunized by intraperitoneal injection of 10^7 spleen cells from C57BL/6 or BALB/c mice. On the 5th day after immunization a test injection of antigen was given, by injecting 5×10^6 allogeneic C57BL/6 or BALB/c cells subcutaneously into the footpad of one hind limb of a mouse in a volume of 0.05 ml, and the same number of syngeneic CBA lymphocytes into the other hind limb. The size of the area of edema was measured 24 h later and a difference of 0.1 mm in the thickness of the right and left limbs was taken as a unit of reaction.

From 2 weeks to 6 months after creation of the chimeras the ability of their lymphocytes to undergo blast transformation in MLC in vitro was investigated. Responding and stimulating cells (irradiated with a dose of 15 Gy) were mixed in the ratio of 1:2 and cultured for 4-5 days, after which the intensity of the reaction was assessed on the basis of ^{3}H-thymidine incorporation. The stimulation index (SI = A/B, where A is the number of counts in the experimental cultures and B the same in the control) was determined.

From 2 weeks to 3 months after induction of chimerism the intensity of the local GVHR was determined under conditions when the lymphocytes of the chimeras were injected into F_1 hybrids. The recipients were given an injection of 15×10^6 splenocytes of the chimeras or control animals in a volume of 0.1 ml subcutaneously into the footpad of one hind limb. The index of the local GVHR was determined 7 days later as the ratio of the weight of the regional popliteal lymph node to the weight of the contralateral node.

To remove cells of donor's origin in a suspension of splenocytes from the chimeras, it was treated in some experiments with CBA anti-C57BL/6 serum and rabbit complement [8], after which the cells were passed through a wadding filter in a solution of low ionic strength, so that dead cells could be removed [15].

EXPERIMENTAL RESULTS

As the writers showed previously, the fraction of cells of donor's (CBA × C57Bl/6F_1) origin in the spleen of the semiallogeneic cyclophosphamide chimeras decreased quickly from 61% (period of chimerism 2 weeks) to 12% (period of chimerism 4 months) [2]. To make clear whether the fall in the level of chimerism in the spleen was accompanied by emergence from the state of tolerance, the immunoreactivity of the chimeras in relation to alloantigens of the donor's line was determined by studying the time course of various parameters.

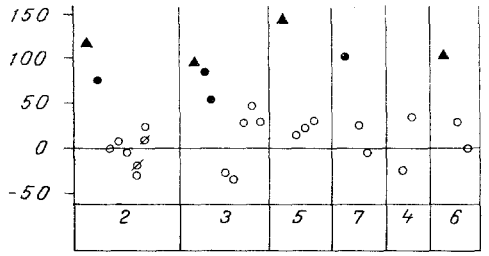

Fig. 3. Areactivity of chimeras' lymphocytes in MLC to C57BL/6 transplantation antigens at different time intervals after induction of tolerance. Horizontal axis — duration of chimerism; vertical axis — response to (CBA × C57BL/6)F_1 cells (in % of magnitude of response to BALB/c cells). Empty circles — response of chimeras' lymphocytes to stimulating C57BL/6 cells; crossed out circles — the same after treatment of chimeras' lymphocytes with line-specific antiserum and complement; filled circles — response of lymphocytes of CBA mice receiving injection of CP to the same antigens as for chimeras; filled triangles — response of intact CBA mice to the same antigens.

Unlike in previous investigations, when allografts of the donor's line were transplanted into chimeras immediately after the induction of tolerance [4, 7], in the present experiments transplantation was carried out later. The time course of rejection of C57BL/6 and BALB/c skin grafts, transplanted into chimeras 2.5 and 4 months after induction of tolerance, is shown in Fig. 1. Rejection of the BALB/c skin graft by the chimeras was complete by the 15th-20th day, whereas the survival of the C57BL/6 grafts in some cases reached 3 months, although most of them were in a state of chronic rejection. This was shown by the long time interval between the beginning and end of rejection. Chronic rejection was particularly marked after transplantation of C57BL/6 skin in the late periods of tolerance (Fig. 1). Thus tolerance to a skin graft of the donor's line in the chimeras was incomplete.

The intensity of the DTH reaction to antigens of donor's origin and to antigens of the 3rd line in semiallogeneic chimeras 2 days after their creation is illustrated in Fig. 2. The level of response of the chimeras to C57BL/6 lymphocytes (Fig. 2:4) was considerably depressed compared with the response of intact CBA and CBA_{cp} (Fig. 2:2, 3). Suppression of the DTH reaction to BALB/c lymphocytes (Fig. 2:7) did not take place in the chimeras by comparison with the control (Fig. 2:5, 6). Some workers consider that the same Lyt 1^+ effector T lymphocytes are responsible for the development of the DTH reaction and for rejection of the grafts [13]. Specific areactivity of the semiallogeneic chimeras in the DTH reaction and the ability of the donor's line not to reject skin and heart grafts for a long time [7], are in agreement with these data. Long-term chronic rejection of a skin graft, observed in chimeras in some cases is evidently linked with the presence of special SK-antigens in the cutaneous epithelium, which are not identical in CBA and C57BL/6 mice, but induce a rejection reaction [14].

The results obtained by recording glass-transformation of the chimeras' lymphocytes in MLC are given in Fig. 3. The chimeras were studied individually at different times after induction of tolerance. In some chimeras there was no response in MLC, whereas in others it was specifically depressed. Areactivity was found throughout the period of observation (6 months) and was unconnected with the presence of F_1 donors' cells in contamination, as was shown by treatment of the splenocytes with CBA anti-C57BL/6 serum and complement.

Immunoreactivity of the chimeras' lymphocytes in vivo was investigated in the local GVHR, using hybrids of the donor's (CBA × C57BL)F_1 genotype and also (CBA × BALB/c)F_1 hybrids as recipients. It follows from Table 1 that specific weakening of the local GVHR was observed only in the late periods (2.5 months) after creation of the chimeras; after 3.5 months, moreover, areactivity of the chimeras had become more marked. Areactivity of the chimeras' lymphocytes in the local GVHR 4 weeks after induction of tolerance was due to contamination with F_1 donor's lymphocytes, as became clear after treatment of the lymphocytes with antiserum and complement. No lowering of the response of the chimeras' lymphocytes to BALB/c antigens was observed but, on the contrary, some stimulation of the reaction was found compared with the response of lymphocytes of the control CBA_{cp} mice. The increase of the response which was

TABLE 1. Intensity of Local GVHR Effected by Spleen Cells of Semiallogeneic Chimeras at Different Times after Induction of Chimerism

Recipients	Time after injection of CP, weeks	Donors of splenocytes								
		No. of group	No. of mice	CBA	No. of group	No. of mice	CBA_{cp}	No. of group	No. of mice	Chimeras
CBA	—	1	32	1,3±0,2	—	—	—	—	—	—
»	—	2	30	5,0±0,4	—	—	—	—	—	—
$(CBA \times C57Bl/6)F_1$	—	—	—	—	9	9	4,4±0,5	8	10	2,6±0,1
»					3					
»	4	—	—	—	4	9	5,2±0,8*	9	6	4,7±0,9*
»	10	—	—	—	5	18	4,8±0,4	10	18	3,7±0,3
»	14	—	—	—	6	7	5,1±0,5	11	5	2,6±0,4
$(CBA \times BALB/c)F_1$	14	—	—	—	7	8	3,5±0,6	12	4	4,8±0,3
»	—	—	—	—	—	—	—	13	4	3,1±0,2*

Legend. Asterisk indicates suspension of splenocytes treated with CBA anti-C57BL/6 serum and complement followed by elimination of dead cells. Values of SI of local GVHR given in this table.

observed was due to the presence of a small number of cells of donor's [(CBA × C57BL/6)F_1] origin in the suspension of chimeras' splenocytes, which led to the development of an additional graft versus host reaction in the (CBA × BALB/c)F_1 recipients. Treatment of the cell suspension with CBA anti-C57BL/6 antiserum and complement before use in the local GVHR abolished the stimulation effect. Thus a fall in the level of the local GVHR in the late stages after induction of tolerance was specific.

Our data showing a sharp decline of immunologic reactivity of the chimeras relative to antigens of the donor's line confirm the hypothesis of the important role of deletion of clones of antigen-recognizing cells in the induction and maintenance of tolerance. However, suppressor cells and serum blocking factor, discovered by the writers previously in cyclophosphamide-induced semiallogeneic chimeras [2, 6], may also be of great importance in this process. In some experiments the areactivity of the chimeras was incomplete. It is possible that suppressor factors under these conditions lead to more complete tolerance to the donor's cells and tissues by the chimeras. Some workers consider that clonal deletion and active suppression are not mutually exclusive factors for the maintenance of immunologic areactivity. Moreover, suppressor cells and serum blocking factor may also facilitate elimination or inactivation of the clone of antigen-reactive cells during induction and maintenance of tolerance [5, 10-12].

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EFFECT OF CHORIONIC GONADOTROPHIN ON COOPERATION BETWEEN SPLENOCYTES FORMING THE PRIMARY IMMUNE RESPONSE

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KEY WORDS: chorionic gonadotrophin; immune response; interleukin-2; prostaglandins.

Chorionic gonadotrophin (CG) is one of the principal hormones of reproduction, and successful implantation and development of the semiallogeneic blastocyst depends on its secretion [2]. Production of the hormone is regularly found in cancer cells [15], and from this standpoint CG has been regarded as a factor preventing rejection of foreign tissue [1]. In all probability the hormone has an immunoregulatory action, but no clear ideas regarding the mechanism of this action have been formulated.

The aim of this investigation was to study the effect of CG on intercellular cooperation processes during the formation of an adoptive immune response, and also to study the identification of certain mediators of this interaction as possible intermediaries in the regulatory action of the hormone.

EXPERIMENTAL METHOD

Experiments were carried out on mature male (CBA × C57BL/6)F_1 mice weighing 18-20 g. Bilateral ovariectomy was performed on some of the animals under ether anesthesia. The period of postoperative rehabilitation was 4 weeks.

During investigation of the action of CG on cooperation between T and B lymphocytes the model suggested previously [13] was adopted, with fractionated splenocytes.

Simultaneously with the antigen (2×10^8 sheep's red blood cells), 4-6 h after lethal irradiation (219.3 mCi/kg) of the recipient mice, they were given an intravenous injection of 10^7 T cells, isolated by filtration of a splenocyte suspension through nylon wadding [12], and 10^7 B cells, isolated by treatment of a suspension of spleen cells with anti-Br-θ-serum [8] and guinea pig complement. The T lymphocytes were identified in the cytotoxic test with anti-Br-θ-serum [9], and B lymphocytes by the EAC-rosette-formation method [4]. On the 5th day the number of antibody-forming cells (AFC) in the animals' spleen was determined by the method in [11].

In the experiments of series I the recipients were given three injections of CG (Moscow Endocrine Factory) on alternate days subcutaneously in a dose of 40 or 200 U, starting from the moment of cell transfer. To determine whether the effect of the hormone was dependent on prostaglandins (PG), in the experiments of series II, parallel with CG, recipient mice received an injection of Voltaren (Pliva, Yugoslavia), an inhibitor of prostaglandin synthetase, in a dose of 3 mg/kg. Both series of experiments were conducted both on castrated and on noncastrated animals. In all the calculations logarithms of the number of AFC were used.

To determine the effect of different doses of CG on interleukin-2 (IL-2) production by the splenocytes, a cell culture (2×10^6 cells/ml), obtained by homogenization of the spleens of

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