It will be clear from Fig. 1 that neither TBHP nor the standard MPh activator bacterial LPS had any effect on the intensity of clearance of the peritoneal cavity from injected bacteria. However, against the background of the deficiency of bactericidal activity of MPh induced by preliminary injection of medium with thioglycollate, the two preparations increased the initially depressed intensity of clearance of the mouse peritoneal cavity by an equal degree. Under the influence of TBHP, just as of bacterial LPS, normalization of the level of clearance of the peritoneal cavity was observed, i.e., the experimentally created deficiency of bactericidal activity of MPh was corrected.

The synthetic antioxidant TBHP was thus found to activate mouse peritoneal MPh by its direct action on them in vitro. After intraperitoneal injection of the same preparation an increase in the number of MPh was found in the peritoneal cavity, and their functional activity also was increased. In mice with a defect of peritoneal clearance induced previously, TBHP led to restoration of the normal level of antibacterial protection. In all tests of activating action on MPh which were studied, this antioxidant was not inferior to the standard MPh activator – bacterial LPS. When TBHP was injected into mice, earlier manifestations of MPh activation were observed than when LPS was injected.

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IMMUNOGENETIC INVESTIGATION OF FAMILIES WITH CLOSE INTERMARRIAGE

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The prospects for the study of genetic control of the human immune response are linked first and foremost with investigation of the HLA-D region of the principal human histocompatibility complex [2]. In order to study the HLA-D region it is necessary to have HLA-D homozygous typing cells (HTC), by means of which not only can HLA-D antigens be detected, but monoclonal typing reagents for class II antigens of the HLA system can also be obtained [2, 3]. The main source from which HTC can be obtained is donors arising from close intermarriages. Although in the USSR no HTC as such are available, on the territory of the Soviet Union there are regions in which offspring of close intermarriages live. One such region is Samarkand. It is worth noting that in the settlements (kishlaks) where such families live, there is a permanently increased prevalence of chronic infectious diseases, so that a genetic basis can be postulated for the disturbance of immunoreactivity found in these patients.

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TABLE 1. Parameters of Cellular Immunity in Members of Families with Close Intermarriages

Group of subjects	Unstimulated BTR, cpm	BTR (IS) PHA, μg/ml 5	PHA, μg/ml 10	PHA, μg/ml 20	Con A, μg/ml 5	Con A, μg/ml 10	Con A, μg/ml 20	Activity of con A-induced suppressor T cells (IR), % (n = 21)	NKC activity (CI), % adults (n=19)	NKC activity (CI), % children (n=12)
Members of families studied	210±32,6	2,27±5,0	3,6±1,5	2,6±0,4	1,8±0,5	1,6±0,5	1,5±0,2	—18,7±9,9	57,1±4,8	49,3±3,2
Control	626±118,3	92,0±18,0	89,7±20,0	72,6±14,9	—	42,1±7,4	—	43,9±7,4	58,6±1,5	59,0±5,2

Legend. Here and in Table 2: n) number of subjects tested; IS) index of stimulation; CI) cytotoxic index.

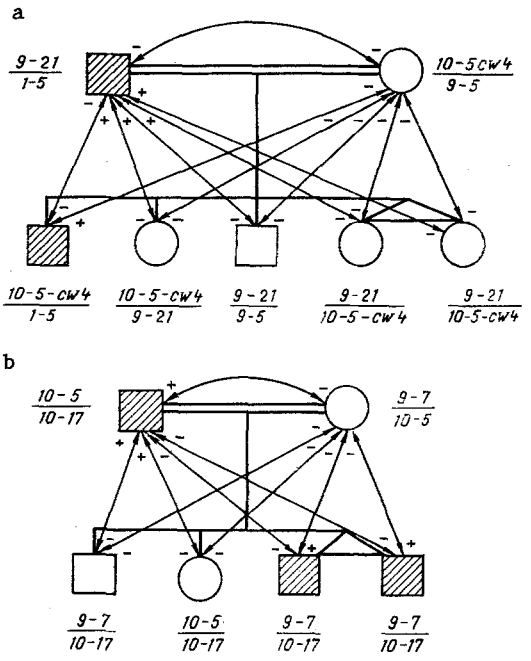

Fig. 1. Detection of HLA-D homozygotes in families with close intermarriages. Shaded symbols indicate probable homozygotes for HLA-D. A plus sign denotes a positive response in MLC, a minus sign − a negative response in MLC. Arrows indicate the direction of the response in MLC.

The aim of this investigation was to discover persons identical with respect to the HLA-D region, and to study the characteristics of the immune status in families with close intermarriages.

EXPERIMENTAL METHOD

Members of 14 Uzbek families with close intermarriages (85 persons) were studied. Lymphocytes were isolated from peripheral blood in a Verografin–Ficoll density gradient (p = 1.077) at 400g for 40 min. Typing for HLA-A, B, and C antigens was carried out in the standard microlymphocytotoxic test. The mixed lymphocyte culture (MLC) was obtained by Alekseev's method [1]. Stimulating cells were treated with mitomycin C (50 μg/ml). The proliferative activity of the T lymphocytes was studied in the blast transformation reaction (BTR), stimulated by phytohemagglutinin (PHA) and concanavalin A (con A) in doses of 5, 10, and 20 μg/ml per 10^6 cells. Activity of nonspecific con A-induced suppressor T cells was determined by the double blast-transformation method [5]. Suppressor T cells were activated by con A (5 μg/ml per $0.5 \cdot 10^6$ cells) for 48 h, after which their activity was tested as reflected in depression of BTR of autologous lymphocytes stimulated by PHA (10 μg/ml per $0.5 \cdot 10^6$ cells). The index of regulation (IR) was calculated by the equation:

TABLE 2. Number of T Cells (E-RFC) and B Cells (EAC-RFC) and Immunoglobulin Levels in Members of Families with Close Intermarriage

Group of subjects tested	E-RFC, %	EAC-RFC, %	Serum immunoglobin concentrations, g/liter		
			IgG	IgA	IgM
Adults	40,0±8,0 ($n = 28$)	20,3±0,8 ($n = 27$)	11,2±2,02 ($n = 33$)	3,0±0,56 ($n = 33$)	1,3±0,22 ($n = 33$)
Children	40,2±6,4 ($n = 42$)	16,3±2,6 ($n = 44$)	10,4±1,56 ($n = 50$)	3,1±0,55 ($n = 50$)	1,1±0,17 ($n = 50$)
Control ($n = 20$)	62,3±9,5	19,5±3,2	15,8±1,8	5,0±2,1	0,9±0,7

$$IR = 1 - \frac{\text{cpm (cells stimulated by con A + cells stimulated by PHA)}}{\text{cpm (unstimulated cells + cells stimulated by PHA)}} \times 100\%.$$

Results with a minus sign were interpreted as a manifestation of the function of activating T cells (Ta). Activity of natural killer cells (NKC) was determined by their cytotoxic action on K562 target cells, labeled with ^{3}H-uridine (3 μCi/ml) in the presence of RNase [4]. In all the reactions the lymphocytes were cultured in medium RPMI-1640 with the addition of human blood group AB (IV) serum and 2 mmoles/liter of L-glutamine at 37°C in an atmosphere with 5% CO_2. The cultures were transferred to filters by means of a microharvester (Flow Laboratories, England). The relative numbers of T and B lymphocytes were determined by the rosette formation test (E-RFC and EAC-RFC). The serum levels of immunoglobulins of the G, A, and M classes was determined by Mancini's immunodiffusion method.

EXPERIMENTAL RESULTS

During cross testing of MLC from members of the families studied, in 13 cases the donor's cells did not stimulate lymphocytes of certain other members of the family in MLC. An example of one such family is illustrated in Fig. 1. By analysis of these results, in the 13 families studied 13 donors who were probably homozygous for HLA-D antigens but at the same time heterozygous for HLA-A, B, and C loci, were revealed.

It will be clear from Tables 1 and 2 that the following particular features were observed in these subjects compared with normal healthy Russian donors (data for Moscow): response to PHA and con A in BTR and suppressor T activity also were depressed. NKC activity was depressed only in children, whereas in adults this parameter, and also the relative number of T and B lymphocytes in the peripheral blood and the immunoglobulin levels were similar to those of Russian donors.

A less marked degree of polymorphism of the class I antigens was thus found in the families studied. It may be expected that among the "unstimulated MLC" cells there were HLA-D-homozygotes. A further investigation of these cells is planned, to include typing, HTC typing and immunochemical analysis with monoclonal antibodies and monomorphic class II determinants. The depression of the functional parameters of the T cell component of immunity which was found may perhaps be the cause of the chronic infectious diseases observed among the population of this region, and connected with the particular pattern of distribution of class II antigens of the HLA system, which are determined by genes associated with human ir genes.

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