SUBPOPULATION STRUCTURE OF HUMAN T LYMPHOCYTES STUDIED WITH MONOCLONAL ANTIBODY CD27

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T cells can be divided into two nonoverlapping subpopulations, which differ from one another both in their antigenic phenotype and in their functional properties. These are $CD4^+$- and $CD8^+$-subpopulations of lymphocytes, mainly associated with helper cells and killer/suppressor cells respectively [7]. Meanwhile, subpopulations of $CD4^+$- and $CD8^+$-cells are not homogeneous, and within them it is possible to distinguish finer sub-subpopulations, which can be found with the aid of antibodies such as CD11b, CD27, CD28, CD29, and CD45RA. The best known antigens in this respect are CD29 and CD45RA, which are distributed mutually exclusively and divide the $CD4^+$-subpopulation into helper-inducer cells and helper-suppressor cells, and the $CD8^+$-subpopulation into true suppressors and precursors of killer cells [8]. Antigen CD28 also divides the $CD8^+$-population into killer and suppressor cells, but the boundary of separation evidently runs somewhat differently from that for antigens CD29 and CD45RA [11]. The character of separation of the subpopulations of $CD4^+$- and $CD8^+$-cells with the aid of antigen CD27 has not yet received adequate study [10]. In particular, we do not know in what proportions the $CD4^+$- and $CD8^+$-populations are divided by antigen CD27, within what limits these proportions may be changed, how the character of division changes depending on the size of the $CD4^+$- and $CD8^+$-subpopulations, and also how this division correlates with subdivision due to other antigens dividing the $CD4^+$- and $CD8^+$-subpopulations. To answer these questions, we obtained monoclonal LP27 antibodies and used them in the investigation described below.

EXPERIMENTAL METHOD

A hybridoma producing monoclonal LT27 antibody (IgG2a) was obtained as a result of fusion of mouse myeloma Sp2/0 cells with spleen cells from BALB/c mice, immunized with peripheral blood lymphocytes from healthy donors [1]. LT27 antibody accumulated in the form of ascites fluid, and was isolated on sepharose-protein A and labeled with biotin [9].

Antigens CD3, CD4, CD20, CD45RA, CD29, CD8, and CD16 were revealed by the use of monoclonal antibodies (McAb) Leu4, Leu3a, Leu16, Leu18 ("Becton Dickinson," USA), 4B4 ("Coulter," USA), and also LT8 and LNK16, obtained by the writers previously [3]. In the two-color immunofluorescence test, cells were treated with McAb labeled with FITC, then with biotinylated McAb and streptavidin, labeled with phycoerythrin. Fluorescence was recorded on a Facscan flow cytometer ("Becton Dickinson," USA). The subpopulation structure was calculated by our previous program [4]. The molecular mass of the CD27 antigen was determined as described previously [2].

A group of 37 children was studied; the group consisted of a control group (n = 24), children without clinical or laboratory signs or symptoms of primary immunodeficiencies, and also a group of patients (n = 4) with combined immunodeficiency (CID) and a group of patients (n = 9) with hypogammaglobulinemia (HGG). Among the patients with CID three had ataxia-telangiectasia and one patient had a severe combined immunodeficiency

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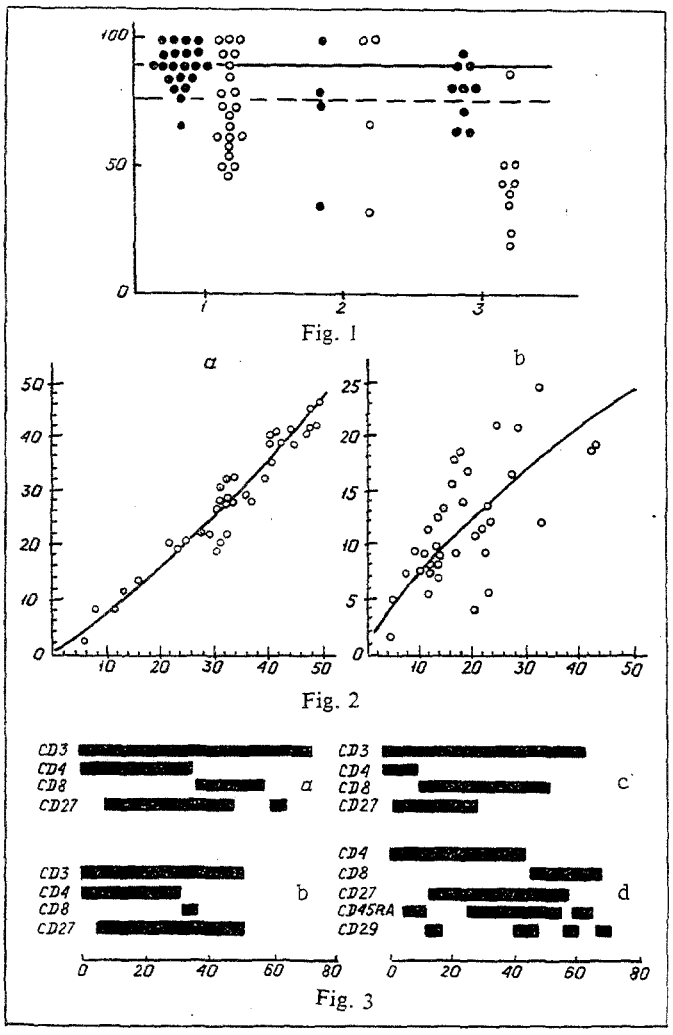

Fig. 1. Comparison of coexpression of antigens CD4 and CD27, and also CD8 and CD27 under normal conditions and in some immunopathologies. Abscissa, groups of subjects; ordinate, fractions of cells coexpressing antigens (in %); filled circles indicate fraction $CD4^+CD27^+/CD4^+$; empty circles – fraction $CD8^+CD27^+/CD8^+$. 1) Normal donors; 2) patients with combined immunodeficiency; 3) patients with hypogammaglobulinemia. Mean values of fractions $CD4^+CD27^+/CD4^+$ and $CD8^+CD27^+/CD8^+$ for normal donors indicated by continuous and broken lines respectively.

Fig. 2. Regression analysis of dependence of number of coexpressing cells on size of $CD4^+$- and $CD8^+$ populations. Abscissa, number of $CD4^+$ (a) or $CD8^+$ (b) cells (in %); ordinate, number of $CD4^+CD27$ (a) or $CD8^+CD27$ (b) cells (in %). Regression coefficients of dependence $y = ax^b$; *a*) $\log(a) = -0.90$, $b = 1.22$, $r = 0.97$; b) $\log(a) = 0.26$, $b = 0.71$, $r = 0.71$.

Fig. 3. Distribution of antigens CD3, CD4, CD8, CD27, CD29, and CD45RA on blood lymphocytes. Abscissa, number of lymphocytes (in %); a, d) normal donor; b) patients with ataxia-telangiectasia; c) patient with hypogammaglobulinemia.

The group with HGG included five patients with congenital agammaglobulinemia and a B-cell deficiency, two patients with agammaglobulinemia with hyper-IgM, and two patients with general variable HGG. The patients were selected by Candidates of Medical Sciences M. N. Yartsev and L. A. Gomes. The diagnosis of primary IDS was made in accordance with WHO recommendations [5].

EXPERIMENTAL RESULTS

A study of reactivity of McAb LT27 showed that LT27 reacted with 65 ± 10% of blood lymphocytes (n = 24) and 15 ± 4% of thymocytes (n = 3) but did not bind with monocytes, neutrophils, erythrocytes, or platelets. LT27 precipitated an antigen with molecular mass under reducing conditions of 55 kilodaltons and nonreducing 110 kilodaltons. According to the results of two-color immunofluorescence, LT27 reacted feebly with B lymphocytes and did not bind with MK cells. On the basis of the results, UcAb LT27 were classed in the CD27 cluster [6].

Investigation of the control group of patients by two-color immunofluorescence showed that the CD27 antigen is present on most T lymphocytes, but distributed among individual subpopulations of T lymphocytes unevenly. Antigen CD27 is represented most strongly on $CD4^+$-cells, 90 ± 8% of which coexpress antigen CD27, and to a somewhat lesser degree, on $CD8^+$-cells, in which only 77 ± 28% of cells carry the CD27 antigen. Among the double negative lymphocytes ($CD3^+CD4^-CD8^-$) 79 ± 12% of cells possessed the CD27 antigen. These ratios were mean values, which varied from donor to donor. For example, in some patients virtually all $CD4^+$-cells carried the CD27 antigen, but the proportion of cells carrying antigens CD^4 and CD27 simultaneously was never below 68%.

Slightly different proportions of separation of the $CD4^+$- and $CD8^+$-subpopulations with respect to antigen CD27 were found in the study of patients with immunodeficiency. Results of comparing the $CD4^+CD27^+/CD4$ and $CD8^+CD27^+/CD8^+$ fractions for the control group of patients with HGG, and also of patients with CID, are shown in Fig. 1. In the first place, in CID and HGG a decrease was observed in the total number of $CD27^+$-cells to 36 ± 14% and 45 ± 13% respectively. The sizes of the $CD4^+CD27^+/CD4^+$ and $CD8^+CD27^+/CD8^+$ fractions in CID varied within wide limits, but their mean values did not differ significantly from the control. In patients with HGG a marked decrease in the $CD4^+CD27^+/CD4^+$ fraction ($p < 0.01$) and an even more marked decrease in the $CD8^+CD27^+/CD8^+$ fraction ($p < 0.001$), which were 80 ± 11% and 45 ± 19% respectively, was observed.

To explain dependence of division of the $CD4^+$- and $CD8^+$-populations with respect to antigen CD27 on the size and relative proportions of the $CD4^+$- and $CD8^+$-subpopulations, regression analysis was used.

For this purpose a combined group of children including a control group and patients with HGG and CID, was examined. The best regression (with the highest coefficient of correlation) was obtained by matching the data by means of a power function $y = ax^b$ (Fig. 2). Dependence of the number of $CD4^+CD27^+$-cells on the number of $CD4^+$-lymphocytes was close to linear (b = 1.2), although some increase was observed in the $CD4^+CD27^+/CD4^+$ fraction with an increase in size of the $CD4^+$ population. Conversely, for the $CD8^+$ population the power index b of the regression dependence of the number of $CD8^+CD27^+$-cells was 0.7, and a definite tendency was observed for the $CD8^+CD27^+/CD8^+$ fraction to decrease with an increase in the total number of $CD8^+$-cells. Good correlation also was found when the number of $CD4^+CD27^+$ cells was compared with the number of $CD4^+$-lymphocytes ($r = 0.97$, $p < 0.001$) and weaker correlation was found in the case of coexpression of antigens CD8 and CD27 ($r = 0.71$, $p < 0.01$). Diagrams of the subpopulation structure of two patients, diametrically opposite to one another with respect to the $CD4^+/CD8^+$-cell ratio, are given in Fig. 3. Patient V suffered from ataxia-telangiectasia and had a ratio of $CD4^+/CD8^+$ of 6.3 (Fig. 3b), whereas patient I. had congenital agammaglobulinemia with a B-cell deficiency, and his $CD4^+/CD8^+$ ratio was 0.3 (Fig. 3c). A diagram of the subpopulation structure of a donor with a normal $CD4^+/CD8^+$ ratio of 2.1 also is given in Fig 3a. Clearly the fraction of $CD4^+$-cells coexpressing antigen CD27 showed little change with a change in the $CD4^+/CD8^+$ ratio, whereas if this ratio was reduced, the fraction of $CD8^+$-cells carrying simultaneously the CD27 antigen, fell appreciably This example also shows that with high values of the $CD4^+/CD8$ ratio the fraction of double negative cells ($CD3^+CD4^-CD8^-$) expressing the CD27 antigen is increased.

To study the relationship between subpopulations discovered on the basis of the presence of antigen CD27 and subpopulations found with the aid of McAb CD45RA and CD29, additional two-color cytograms of fluorescence were obtained for five donors. Lymphocytes of each donor were stained with the following combinations of McAb:

CD27 and CD4, CD27 and CD8, CD27 and CD45RA, CD29 and CD4, CD29 and CD8, CD29 and CD45RA, CD45RA and CD4, and CD45RA and CD8. The subpopulation structure obtained for one of the donors, allowing for simultaneous expression of the five antigens studied, is illustrated in Fig. 3d. The diagram shows that antigens CD29 and CD45RA are present on $CD4^+$- and $CD8^+$-cells, both carrying the CD27 antigen and not expressing it. Similar results also were obtained for the other donors studied. Thus the separation of $CD4^+$- and $CD8^+$-subpopulations by antigen CD27 differs from separation based on expression of antigens CD29 and CD45RA.

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