USE OF MONOCLONAL ANTIBODIES AGAINST RAT IMMUNOGLOBULIN LIGHT CHAINS IN RADIOIMMUNOASSAY OF DIFFERENTIATED MOUSE LYMPHOCYTE ANTIGENS

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Monoclonal antibodies (MCA) of rats immunized with mouse lymphocytes are widely used to study the membrane markers of lymphocytes [4-6]. Interaction between rat MCA and mouse cells is usually detected by means of rabbit antibodies against rat immunoglobulin (Ig), labeled with fluorescein isothiocyanate, ^{125}I, or enzyme. The evaluation of this reaction is made substantially more difficult by the fact that rabbit antibodies against rat Ig, which cross-react with mouse Ig, interact directly with mouse B lymphocytes carrying Ig. To abolish this reaction, the antibodies against rat Ig have to be adsorbed by mouse Ig, and this considerably depresses their activity. This difficulty can be avoided if, not rabbit serum, but mouse serum or mouse MCA against rat Ig is used as the anti-Ig. The strongest response to rat Ig is given by mice of the SJL strain [6].

The aim of the present investigation was to obtain mouse MCA against the L-chain of rat Ig and to use these MCA to detect mouse surface antigens.

EXPERIMENTAL METHOD

BALB/c mice were obtained from the "Stol'bovaya" Nursery, Academy of Medical Sciences of the USSR, and SJL mice and Lou/wsl rats were obtained from the nursery of the All-Union Oncologic Scientific Center, Academy of Medical Sciences of the USSR. Rat Ig light chains were obtained by the method described in [2]. SJL mice were immunized intraperitoneally with 70 μg of rat Ig light chains in Freund's complete adjuvant, followed 14 days later by a similar injection in physiological saline. After 4 days spleen cells of the immune mice were hybridized with 10^7 cells of mouse myeloma P4-X63-Ag 8.653 (X63) [1]. The presence of antibodies against rat Ig L-chains in the culture fluid (CF) was estimated by the method described previously [8]. Cells in cultures producing antibodies against rat Ig L-chains were cloned twice by the limiting dilution method, using macrophages as the "feeder" [7]. Seven independently obtained clones synthesized MCA, which reacted either with the whole rat Ig molecule irrespective of the allotype of the L-chains or with L-chains and Ig carrying only the RL-2 allotype, or only with the L-chain but not with the whole Ig molecule. In this investigation three variants of MCA of the first type were selected, i.e., those reacting with whole Ig of any allotype. All three variants of MCA were isolated from CF by affinity chromatography on a column with rat IgG immobilized on CNBr-activated Sepharose 4B (from Pharmacia, Sweden). Elution was carried out with 0.2 M glycine buffer (pH 2.5) containing 0.15 M NaCl. The isolated MCA were labeled with ^{125}I [3] to a specific activity of 10^6 counts /μg protein.

Rat MCA C1-9 (μ, κ) and C4-20 (μ, κ) against antigens of mouse T suppressors, obtained previously [4] by the writers, and also rat anti-Lyt-1 and anti-Lyt-2 [6], generously provided by R. G. Vasilov, were used as CF. For radioimmunoassay 10^6 lymphocytes were incubated in 100 μl CF for 1 h at 4°C in V-shaped wells in 96-well plates (from Linbro, England), and after rinsing three times, in 50 μl of ^{125}I-labeled MCA for 1 h at 4°C, in a dose of 10^5 counts per well. The three washed cells were transferred to flasks and their radioactivity counted on a Gamma 400 gamma-spectrometer (Beckman, USA). The same cells treated with control CF (CCF) of melanoma X63 and ^{125}I-labeled MCA, were used as the negative control. The binding index was expressed as

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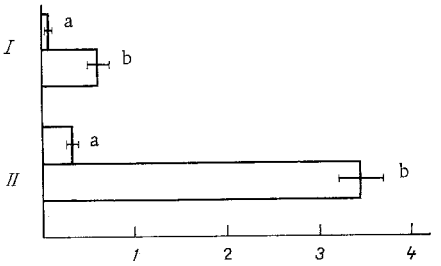

Fig. 1. Comparison of interaction of mouse MCA against rat Ig L-chains with BALB/c mouse and Lou/wsl rat spleen cells. Binding of ^{125}I-labeled L1G9 (I) and L3E8 (II) MCA with BALB/c mouse (a) and Lou/wsl rat (b) spleen cells. Here and in Figs. 2 and 3: abscissa, incorporation of ^{125}I (in cpm · 10^{-3}).

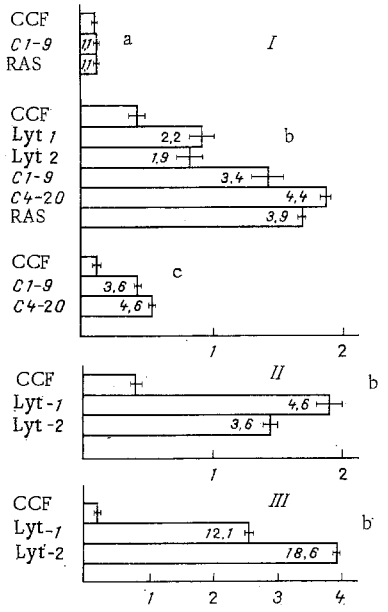

Fig. 2. Demonstration of response of antibodies of differential markers of mouse lymphoid cells by MCA against rat Ig L-chains. Binding of ^{125}I-labeled L1G9 (a), L3E8 (b), and L2B2 (c) MCA with spleen (I), lymph node (II), and thymus (III) cells of BALB/c mice treated beforehand with CCF, RAS, or MCA to C1-9, C4-20, Lyt-1, and Lyt-2 antigens. Here and in Fig. 3, numbers in columns show binding indices.

the ratio of activity of the label in the experimental and control samples. Rat antiserum (RAS) against mouse lymphocytes was collected from rats whose spleens were used for hybridization during preparation of the C1-9 and C4-20 MCA.

Properties of MCA produced by three independent hybridomas (L1G9, L2B2, and L3E8) were investigated. First, their ability to react with lymphoid cells in the direct radioimmune test was studied. MCA L1G9 and L3E8, labeled with ^{125}I, bound with rat spleen cells 4.1 and 9.1 times more effectively, respectively, than with mouse spleen cells (Fig. 1). These data show that MCA reacting with rat Ig bind with mouse splenocytes only at a low level of nonspecific adsorption.

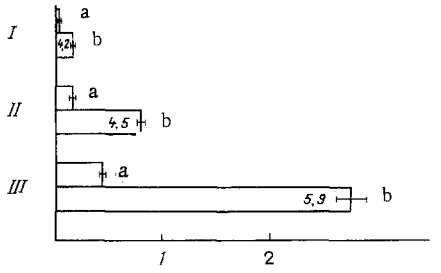

Fig. 3. Dependence of binding of L3E8 MCA against rat Ig L-chains on dose of MCA. BALB/c mouse spleen cells preincubated with CCF (a) or C1-9 MCA (b), treated with ^{125}I-labeled L3E8 MCA in doses (per well) of: $5 \cdot 10^4$ (I), 10^5 (II), and $5 \cdot 10^5$ (III).

EXPERIMENTAL RESULTS

In the next experiments the possibility of using the MCA thus obtained in an indirect test for detecting binding of rat MCA, directed toward surface antigens of mouse cells, was investigated. Rat MCA against the well studied T-cell antigens Lyt-1 and Lyt-2, and also C1-9 and C4-20 MCA, reacting with limited populations of T and B lymphocytes, were selected. RAS was used as the positive control. It will be clear from Fig. 2 that L3E8 and L2B2 MCA effectively bind with lymphocytes treated beforehand with rat MCA. MCA against rat L-chains react equally effectively with rat MCA irrespective of their class – IgG (MCA against Lyt-1 and Lyt-2) or IgM (C1-9 and C4-20) (all four MCA have light chains of the $\varkappa$-type). When anti-Lyt-1 and anti-Lyt-2 MCA were used, the levels of binding of ^{125}I-MCA L3E8 by lymph node, spleen, and thymus cells differed: Spleen cells and, in particular, lymph node cells bind anti-Lyt-1 MCA more efficiently than anti-Lyt-2; thymocytes, on the other hand, bind anti-Lyt-2 MCA more effectively than anti-Lyt-1 (Fig. 1). The binding index for the reaction of spleen cells with C1-9 MCA (3.4-3.6) and C4-20 MCA (4.4-4.6) was almost independent of which labeled MCA were used – L3E8 or L2B2. Figure 2 also shows that MCA against T suppressors bind with splenocytes 1.4-2.3 times more effectively than anti-Lyt-1 and anti-Lyt-2 MCA. By contrast with L2B2 and L3E8 MCA, binding of ^{125}I-labeled L1G9 MCA was indistinguishable from the negative control whether the spleen cells were treated with C1-9 MCA or with RAS (Fig. 1).

L3E8 MCA were equally effective as "developers" of antibodies when used in doses of between $5 \cdot 10^4$ and $5 \cdot 10^5$ counts: On treatment of spleen cells with C1-9 MCA in the above doses the binding index showed little change despite a substantial change in the absolute values of MCA binding (Fig. 3).

The mouse MCA obtained against rat Ig L-chains can thus be used to detect binding of rat antibodies (including monoclonal) with mouse lymphocytes. L3E8 MCA react with rat MCA irrespective of the class of heavy chain, by interacting with the light chains of $\varkappa$-type. Since 95% of all rat Ig light chains belong to the $\varkappa$-type [3], L3E8 is a virtually universal reagent. Despite good reactivity relative to L-chains and whole rat Ig, and also to rat spleen cells in the direct test, L1G9 MCA in the indirect radioimmune test virtually do not bind at all, and this may perhaps be explained by low affinity of the L1G9 MCA or their affinity for the L-chain determinant, which is only weakly expressed in the antigen–antibody complex.

The significant advantage of mouse antibodies against rat Ig over rabbit antibodies in this case is as follows. First, mouse antibodies, unlike rabbit, do not in fact react with native mouse lymphoid cells, expressing membrane Ig. In the controls low figures for binding of the label were obtained, and adsorption of antibodies by mouse Ig was unnecessary. Second, induction, not of ordinary antibodies, but of monoclonal antibodies against rat Ig L-chains enables not only binding of rat MCA with mouse cells to be identified, but also allotypic determinants (RL-1 and RL-2) of these MCA, and this may be important for immunogenetic and population analysis of the distribution of Ig genes within the species [3].

Mouse MCA which the writers have now obtained against rat Ig L-chains are being used intensively to study T-cell markers and to investigate Ig genes.

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MONOCLONAL ANTIBODIES AGAINST DIFFERENTIATING ANTIGENS OF HUMAN THYMOCYTES

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It has recently been found that monoclonal antibodies can be used to identify different levels of lymphoid differentiation and individual subpopulations of T lymphocytes. Common T-cell antigens [2, 4, 6], antigens of T-suppressors/cytotoxic cells [4, 7], of T-helpers/inducers [5, 7, 9], thymocytic antigens [8, 9], and so on, have been found. These antibodies are used both to study subpopulations and stages of differentiation of T cells and also for the immunodiagnosis of leukemia and lymphosarcoma.

The aim of this investigation was to obtain hybridomas producing monoclonal antibodies against differentiating thymocyte antigens, suitable for immunodiagnosis of leukemia and lymphosarcoma in man.

EXPERIMENTAL METHOD

Hybridomas were obtained by the method in [4]. BALB/c mice were immunized with human embryonic thymus cells treated with monoclonal IKO-1 antibodies against DR-antigens and IKO-5 antibodies against HLA antigens. One mouse received nine intravenous injections each of $2 \cdot 10^7$ cells in the course of 8 months, another received 3 injections in the course of 4 months. Splenocytes were fused with P3×63Ag653 cells with the aid of 50% polyethylene glycol with a molecular weight of 1500. After growth in selective medium and screening, the producing hybridomas were cloned twice by the limiting dilution method on feeder consisting of splenocytes and thymocytes from BALB/c mice. The antibodies were screened and tested in the indirect surface immunofluorescence test (IFT). The IFT with thymocytes was carried out on the cell suspension, and the other cells were attached to the glass by means of 50 μg of poly-L-lysine. The thymocytes were fractionated in an 8-step bovine serum albumin (BSA) gradient [3]; 10^9 thymocytes in 17% BSA solution were layered above a 19-33% BSA gradient and centrifuged at 1000g for 35 min at 4°C. More than 65% of the cells were contained in fractions 4-6. Early precursors of T cells and medullary thymocytes were sedimented in fractions 1-3, and cortical thymocytes in fractions 4-6 [3]. The fetal thymus was obtained during spontaneous abortion, the child thymus during open heart operations.

EXPERIMENTAL RESULTS

Two hybridomas producing monoclonal antibodies reacting with thymocytes from three 24-week-old fetuses and 9 children aged 5-15 years were obtained. Monoclonal IKO-11 antibodies reacted with 75.4 ± 1.6% of thymocytes, IKO-10 with 2.5 ± 0.06% (Table 1). IKO-10 antibodies reacted with 40-60% of thymocytes from fraction 1 of the BSA gradient (Fig. 1). Fractions 2 and 3, in which medullary thymocytes also were sedimented,

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