ROLE OF LFA-1 ANTIGEN α-CHAIN IN THE LYMPHOCYTE ADHESION INHIBITION PHENOMENON STUDIED WITH ICO-11 MONOCLONAL ANTIBODIES IN VITRO

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On the surface membrane of cells there are many macromolecules which are essential in order to maintain the normal activity of the cell and the performance of its functions. Monoclonal antibodies (MAB) are a useful tool for the study of such macromolecules. It has been shown that MAB can bind with the active center of a molecule and block its function. If MAB, in the absence of complement, block a certain function of immunocompetent cells, antigens against which these particular MAB are aimed, are called functionally associated antigens. These include the E-receptor, the T3/Ti complex, antigens LFA-1 and LFA-3, antigens of the chief histocompatibility complex of the I and II classes, etc. [7, 9]. In the Laboratory of Clinical Radioimmunology (Director, Professor Z. G. Kadagidze), Institute of Clinical Oncology (ICO), All-Union Oncologic Scientific Center, Academy of Medical Sciences of the USSR, ICO-11 MAB aimed against lymphocytic functionally associated antigen (LFA-1) have been obtained [2]. It has been shown that ICO-11 MAB of the IgG3-isotope block activity of natural killer cells, the blast transformation of lymphocytes reaction, cytotoxicity of T killer cells induced in a 7-day mixed lymphocyte culture, and also E-rosette formation, although the effect of ICO-11 on inhibition of lymphocyte adhesion has not previously been studied [1, 2].

The aim of the present investigation was to study the effect of ICO-11 MAB on the lymphocyte adhesion inhibition (LAI) test on patients with breast cancer in vitro.

EXPERIMENTAL METHOD

The culture medium of a hybridoma ICO-11 in dilutions of 1/2, 1/4, 1/8, 1/16, 1/32, and 1/64 was used as the source of antibodies in the experiments. Human peripheral blood lymphocytes and also extracts of tumor and normal tissue were obtained by methods described previously [4, 6].

To study the effect of MAB in the LAI test [5] 0.1 ml of serial dilutions of ICO-11 MAB was added to the wells in 96-well plastic micropanels (No. 3040, from Falcon Plastics, USA) (culture fluid of X63.Ag 8.653 myeloma cells in the same dilutions was used in the control) and to each well was added 0.1 ml of a suspension of a patient's lymphocytes (a healthy blood donor in the control) in a concentration of $2 \cdot 10^6$ cells/ml in medium 199 with 15% embryonic calf serum, inactivated by heating to 56°C for 30 min. The lymphocytes were incubated with MAB for 30 min (37°C, 5% CO_2), after which 0.05 ml of tumor tissue extract (normal tissue extract in the control) in a concentration of 1 mg/ml as protein, was added to the culture wells (0.2 mg per well). The panels were then reincubated for 1.5 h, after which the number of nonadherent cells in each well was counted by the method in [3]. The results were assessed by means of the LAI index, calculated by the formula $[(a - b)/b] \cdot 100\%$, where a is the mean number of nonadherent cells in the experimental samples, and b the same in control samples.

A negative LAI index signifies intensification of lymphocyte adhesion. The T-cell nature of production of the lymphocyte adhesion inhibition factor (LAI factor) in this particular

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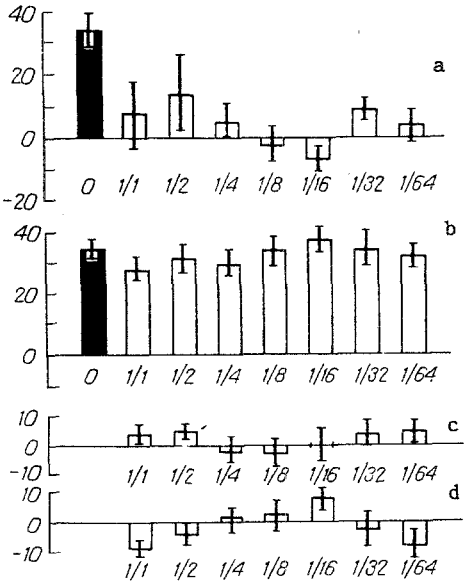

Fig. 1

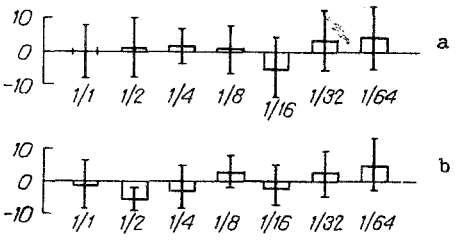

Fig. 2

Fig. 1. Effect of ICO-11 MAB on reaction in LAI test. Abscissa, dilution of ICO-11 or of control supernatant; 0) initial LAI reaction; ordinate, LAI index (in percent). a) Patients' lymphocytes + tumor extract + ICO-11 MAB; b) patients' lymphocytes + tumor extract + control supernatant; c) patients' lymphocytes + normal extract + ICO-11; d) patients' lymphocytes + normal extract + control supernatant.

Fig. 2. Effect of ICO-11 MAB on spontaneous adhesion of lymphocytes from healthy blood donors. Abscissa, dilution of ICO-11 MAB or of control supernatant of X63.Ag 8.653 myeloma cells; ordinate, LAI index (in percent). a) Donors' lymphocytes + ICO-11 MAB; b) donors' lymphocytes + control supernatant.

test system was demonstrated by the writers previously [5].

Spontaneous lymphocyte adhesion was studied by the method in [3, 5], which is identical with the LAI test, but without addition of extracts of tumor and normal tissues. The statistical significance of the results was estimated by Student's t test.

EXPERIMENTAL RESULTS

It will be clear from Fig. 1a that addition of MAB in a dilution of 1:4 or higher, significantly ($p < 0.01$) blocked the reaction in the LAI test. An increase in scatter of the results when high MAB titers were used was evidently connected with nonspecific adsorption of protein on the cells and the plastic surface, which was observed in the LAI test previously when high concentrations (as protein) of antigen were used [5]. Addition of control supernatant X63.Ag 8.653 to the test system in the same titers as MAB had no effect on the original reaction in the LAI test (Fig. 1b).

Data on the effect of ICO-11 MAB and of the control X63.Ag 8.653 supernatant on adhesion of patients' lymphocytes in the presence of normal tissue extract are given in Fig. 1c, d. Neither MAB nor the control supernatant caused any significant changes in the parameters in the samples tested. Thus ICO-11 MAB blocked the reaction in the LAI test evidently by their direct influence on the immunologic reaction, on the process of binding of T lymphocytes, producing the LAI factor of the tumor-associated antigen, and not through their influence on spontaneous adhesion of patients' lymphocytes in the presence of the corresponding tissue extract.

The results of experiments to study the effect of ICO-11 MAB on spontaneous adhesion of normal human lymphocytes are given in Fig. 2. They show that ICO-11 MAB and the control supernatant, in the dilutions studied, had virtually no effect on the adhesive properties of lymphocytes from healthy blood donors. These results are in agreement with data in the literature according to which the α-chain of the LFA-1 antigen is not involved in the process of adhesion of cells to a plastic surface [8].

This investigation thus showed that ICO-11 MAB block the response of patients' lymphocytes to tumor tissue extract in the LAI test in vitro, but have no effect under these circumstances on the response of patients' lymphocytes to normal tissue extract, or on spontaneous adhesion of normal human lymphocytes. Consequently, the results indicate that ICO-11 MAB block the binding of T cells, producing the LAI factor, with tumor-associated antigen. The ability of ICO-11 MAB to block activity of different subpopulations of immunocompetent cells was described previously in other immunologic tests. ICO-11 MAB blocked activity of natural killer cells of healthy blood donors, aimed against K-562 and Molt-4-4 target cells, and also depressed activity of cytotoxic T lymphocytes, induced in a 7-day mixed lymphocyte culture [2]. It has also been shown that ICO-11 MAB have a dose-dependent inhibitory influence on the lymphocyte blast transformation response to phytohemagglutinin and that they inhibited E_H-rosette formation in healthy blood donors [2].

Determination of the molecular weight of the antigens by immunoblotting showed that ICO-11 MAB revealed a polypeptide with mol. wt. of 180 kilodaltons from lysates of human blood mononuclear cells and thymocytes [2]. The spectrum of cells expressing the antigen revealed by ICO-11 MAB, the blocking of natural killer-cell activity [1] and of the lymphocyte blast transformation reaction, and the molecular weight of the antigen revealed indicate that ICO-11 MAB are aimed against the α-chain of a functionally-associated lymphocyte antigen [2]. Blocking of the reaction of ICO-11 MAB in the LAI test thus indicates a possible role of the α-chain of the functionally associated antigen in binding of the tumor-associated antigen by T cells in the phase of induction of the lymphocyte adhesion inhibition reaction.

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DYNAMICS OF ENDOGENOUS DEOXYRIBONUCLEASE ACTIVITY OF MOUSE THYMUS AND SPLENIC LYMPHOCYTE NUCLEI DURING THE IMMUNE RESPONSE

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Cell nuclei of animals and man contain various nucleases, among them endogeneous deoxyribonucleases (endo-DNases) [12]. The functions of these enzymes in higher eukaryotes have not yet been explained. However, data obtained on prokaryotes show that endonucleases take part in various genetic processes, mainly DNA recombination and repair. Most probably the nucleases of the cell nucleus must also perform similar functions. For endonucleases of one type, associated with chromatin, namely apurine/apyrimidine endonucleases, their participation

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