Monoclonal Antibody against A, Lewis d Antigen Produced by the Hybridoma Immunized with a Pulmonary Carcinoma

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ABSTRACT

A monoclonal antibody (CALed) against a human pulmonary squamous cell carcinoma line was cytotoxic to the line but did not react to an autologous B-lymphoblastoid line. Although the antibody was thought to be cancer specific, principally on the basis of this evidence, the antibody actually had the A, Lewis d (Le⁹) specificity. It reacted with approximately 2% of the random donor T-lymphocytes and with all six lymphocytes from donors who were A, Le⁹ type without reacting to lymphocytes of any other type. The monoclonal antibody reactivity was also absorbed out by A, Le⁹ red blood cells but not by red cells of other types. We conclude that the A, Le⁹ antigen had been synthesized by the pulmonary carcinoma lines but not by the autologous lymphoblastoid line, resulting in disparity for this antigen. Since the combination A, Le⁹ only occurs in 2% of the population, it is difficult to distinguish this type of antibody from tumor-specific antibodies.

INTRODUCTION

The monoclonal antibody technology of Kohler and Milstein (7) has been reported to produce specific antibodies to tumor cells (1, 3, 4, 6, 8, 9, 12, 14). Very highly specific antibodies that have hitherto not been possible to make in heterologous immunization are now possible by this method. However, we wish to report here how an apparently highly tumor-specific antibody has now been shown to be directed against the A, Le⁹ antigen. Originally, the monoclonal antibody was found to react only to the immunizing pulmonary carcinoma cells but not to the lymphoblastoid line from the same donor (5). Because these 2 tissues can be assumed to share the same histocompatibility antigens, the monoclonal antibody was thought to be tumor specific. It was also noted that the antibody reacted to one other colon cancer line. When the antibody was tested with lymphocytes from 100 persons, it reacted with 2% of the donors' cells. As reported here, when these reactions were studied further, it was discovered that the 2 cells had the rare combination A, Le⁹ and that in fact the antibody has this specificity.

MATERIALS AND METHODS

Cell Line. The pulmonary tumor cell line, designated UCLA-So-P3, hereafter called P3, is a squamous cell carcinoma of the lung derived from a 53-year-old white male of RBC type A. P3 expresses HLA antigens A2, A10, BW44, DR2, and DR5. The autologous B-lymphoblastoid line (PL3) was established in culture from the patient's peripheral lymphocytes by infection with Epstein-Barr virus.

Immunization and Fusion. A 10-week-old BALB/c mouse was immunized by i.p. injection with 10⁷ P3 tumor cells 1 week apart. Three days after the last inoculation, sensitized spleen cells (5 x 10⁶ cells) were harvested, mixed with 1 x 10⁶ S194 myeloma cells, and fused in 50% polyethylene glycol 1500. The fused cells were distributed in microculture plates (6 x 10⁵ cells/well) and cultured for 2 weeks in Roswell Park Memorial Institute Tissue Culture Medium 1640 with 10% fetal calf serum containing hypoxanthine (13.6 mg/liter), aminopterin (0.176 ml/liter), and thymidine (3.88 ml/liter) to select lymphocyte-myeloma hybrid cells. The hybrid cells were grown in culture medium containing hypoxanthine and thymidine for another week. After the antibody detection assay, the positive hybridomas were cloned by the limiting dilution method.

Microdroplet Lymphocytotoxicity Test. Cytotoxic reactivity of monoclonal antibody was carried out by the complement-dependent lymphocytotoxicity test (13). Briefly, 1 μl of antibody was incubated with approximately 1500 target cells for 1 hr, followed by 2-hr incubation in rabbit complement. The incubation temperature with both antibody and complement was uniformly 25°C. The percentage of dead cells was counted by dye exclusion.

T-Lymphocyte Panel. One hundred T-lymphocytes from normal individuals isolated by nylon wool (2) were used as a panel. They were typed for HLA-A, -B, and -C antigens (13). Le⁹ and Le⁶ types were determined by RBC agglutination (Ortho Diagnostics). Donors whose RBC and serum were Le⁹ and Le⁶ were separated into secretors (Le⁹) and nonsecretors (Le⁶) by using their saliva in a hemagglutinin inhibition test. Anti-A, anti-B, and anti-H (Ulex europeus) were used for these tests. A, and A⁰ types were determined by RBC agglutination with anti-A, (Dolichos biflorus) and anti-H.

Four T-lymphocytes from A, Le⁹ individuals were then deliberately selected for further investigation.

Aliantibody to A, Le⁹. Two alloantisera to A, Le⁹ (L6934 and L0884) were obtained from, multiparous women. The specificity of these 2 antisera was carefully monitored by a huge number of cells as described before (11). RBC Absorption. RBC were washed at least 7 times in Hanks' balanced salt solution, and then packed RBC were applied for absorption with an equal volume of CALed at 0°C overnight.

RESULTS

The monoclonal antibody produced was cytotoxic to P3 lung carcinoma cells to a dilution of 1:256. No reactivity was observed to PL3 which was an autologous B-lymphoblastoid established line from the same patient (Chart 1). Lymphocytotoxic reactivity of the monoclonal antibody to the panel of lymphocyte donors, typed previously for ABO and Lewis status, is shown in Chart 2. Six T-lymphocytes from the individuals with A, Le⁹ phenotype showed cytotoxicity at a dilution of 1:128. T-Lymphocytes from A, Le⁶, B Le⁹, and O Le⁹ individuals were negative to this monoclonal antibody. In
addition, T-lymphocytes from A,B Leb and from RBC type A individuals regardless of Lewis type were not killed by the antibody.

Cytotoxic reactivity of CALed and 2 alloantibodies (L6934 and L0884) to different tumor cell lines are shown in Table 1. P3 and HT29, a colon carcinoma line, were killed by the monoclonal antibody. Two lines were also killed by L6934 and L0884 which were cytotoxic alloantibodies to A, Leb.

The results of RBC absorptions with different phenotypes are shown in Table 2. The reactivity to P3, the original immunizing cell, was absorbed out by A, Leb RBC but not by A, Leb, A, Leb, and A,B Leb RBC.

The monoclonal antibody did not agglutinate the RBC with A, Leb but hemolyzed them readily with rabbit complement.

**DISCUSSION**

If an antibody reacts to tumor cells and does not react to lymphocytes from the same donor, the antibody has been assumed to be tumor specific. This assumption would seem reasonable, particularly if the antibody does not react to various other tumors or tissues from other people. The findings described here show that, even with these safeguards, one cannot conclude that the antibody is tumor specific in all cases. In this particular example, it was only by testing a large panel of lymphocytes that the chance observation was made that only 2 of 100 lymphocytes reacted with the antibody. Another interesting feature shown by these tests is that some antigens may be expressed by the tumor but not by other cells such as lymphoblasts. The A, Leb antigen is apparently synthesized by the tumor but not by the lymphoblast. On the other hand, lymphocytes from peripheral blood contain the A, Leb antigen since it is absorbed from the serum (10). This then accounts for why the tumor cell reacted with the antibody whereas the lymphoblast line from the same donor did not. The single other cell with which the antibody reacted, a colon cancer cultured line, was also shown to have the A, Leb antigen.

The 3 main lines of evidence indicating that the antibody is indeed against A, Leb are as follows. (a) It was possible to locate 6 persons who were A, Leb and all 6 had lymphocytes which were killed by this antibody to a 1:128 dilution. Lymphocytes from other persons who were not A, Leb were not killed by the antibody. (b) The monoclonal antibody could be absorbed by A, Leb RBC but not by other RBC. Thus, the tumor-
specific activity could be removed by only the A, Le" cells but not by other RBC. And (c) although the monoclonal antibody did not agglutinate A, Le" RBC, it could be shown to have reacted with the cells by the Coombs reaction and hemolytic test.

REFERENCES


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