Further Studies on a Hybrid Cell-Surface Antigen Associated with Human Chromosome 11 Using a Monoclonal Antibody

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Abstract—*A monoclonal antibody has been obtained that recognizes an antigen encoded by human chromosome 11. We present evidence that this monoclonal antibody recognizes the same or a similar antigenic activity as that previously called a_1. Genetic information necessary for a_1 expression and recognition by the monoclonal antibody both map to $11p13 \rightarrow 11pter$. Mutants that have lost a_1 are no longer recognized by the monoclonal antibody. The macroglycolipid fraction of human erythrocyte membranes which contains the a_1 antigenic activity is able to convert antigen-negative Chinese hamster ovary cells into cells which are killed by the monoclonal antibody plus complement.*

INTRODUCTION

Previous work from this and other laboratories has identified several different human cell-surface antigens coded for by human chromosome 11 using human–rodent somatic cell hybrids (1–7). These antigens have been identified utilizing species-specific antisera. The ability to prepare monoclonal antibodies to these antigens makes more precise analysis possible.

In this paper, we describe an IgM monoclonal antibody that recognizes an antigen coded for by chromosome 11. This antigen maps on $11p13 \rightarrow 11pter$ and, using mutant and glycolipid conversion analyses, we show that this monoclonal recognizes the antigen activity we previously designated a_1 (also referred to as SA11-1 and S1).

489

MATERIALS AND METHODS

Cell Lines and Cell Culture. The following cell lines were used: CHO-K1, a Chinese hamster ovary cell line; J1, a human–Chinese hamster ovary hybrid which contains human chromosome 11 as its only human chromosome (8); J1–7, 11, 23, 35 (9, 10), subclones of J1; a panel of independently isolated human–Chinese hamster hybrids containing different combinations of human chromosomes; and HeLa and a variant HeLa line. These cells were cultivated in F12FC5 (11).

For hybridoma production the human cell lines UM-SCC-1, a squamous cell carcinoma line established at the University of Michigan (12), SK-MEL-28, a malignant melanoma line (13), WI-38, a normal human fibroblast line, and SK-OV-3, an ovarian adenocarcinoma line, were used. SK-MEL-28, WI-38, and SK-OV-3 were all obtained from the tumor cell bank at the Sloan-Kettering Institute. Human cell lines were maintained in Eagle's minimum essential media (MEM) supplemented with 2 mM glutamine, 1% nonessential amino acids, 100 IU/ml penicillin, 100 μg/ml streptomycin, and 15% fetal bovine serum. NS-1 mouse myeloma cells (14) were obtained from B. Grossman and were cultured in RPMI 1640 medium.

Hybridoma Isolation and Monoclonal Antibody Production. BALB/c mice were immunized intraperitoneally with 30 million viable cells of the human squamous cell carcinoma line UM-SCC-1. At 8 and 30 weeks after immunization, the mice were given booster inoculations of 3 million cells. Three days after the last immunization, 10^8 immune spleen cells were fused to 10^7 NS-1 myeloma cells (14) with 30% polyethylene glycol 1000 (Koch-Light Laboratories, Ltd., Berks., England). Fused cells were plated in petri dishes in complete Iscove's medium containing 20% FCS, 36 mM NaHCO$_3$, and 50 μg/ml garamycin. After a 48-hr incubation at 37°, cells were plated in 96-well plates (Corning 25860) in complete Iscove's medium (Gibco Cat. No. 430-2200, Grand Island, New York) containing 1×10^{-4} M hypoxanthine, 1.6×10^{-5} M thymidine, and 4×10^{-7} M aminopterin (HAT) (15). The medium in each well was changed twice before testing for antibody production. Supernatant from wells containing hybrids was assayed for specific antibody by a mixed hemadsorption assay (13) against UM-SCC-1 cells. Positive supernatants were then tested against a panel of human cell lines including UM-SCC-1, a fibroblast line, a melanoma, and an ovarian carcinoma. Hybridomas giving unique patterns of reactivity were selected, subcloned, and the resulting monoclonal antibody-containing supernatants were tested extensively for reactivity to human malignant and normal cell lines of different histologic types. Established hybridoma cultures were maintained in Iscove's medium. Monoclonal antibody E7 is an IgM antibody that binds to nearly all human cells.

E7 Culture Supernatant (%)
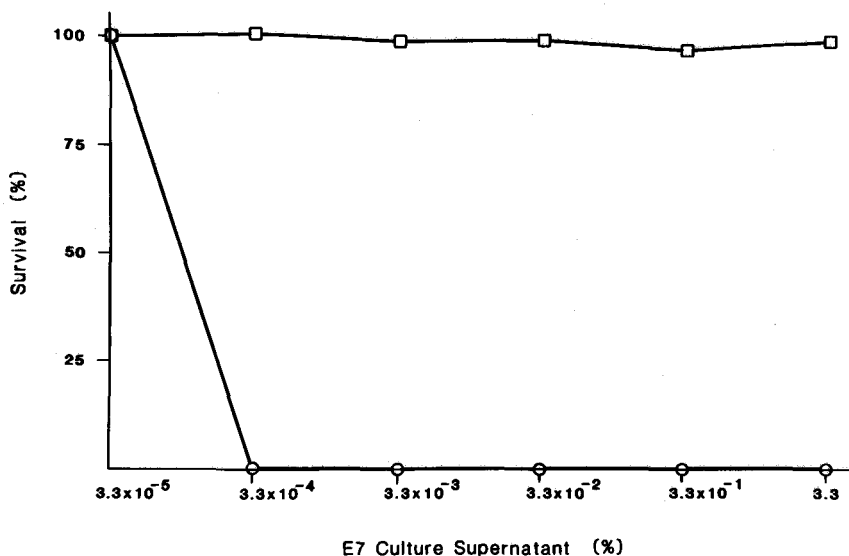
Fig. 1. Percentage survival in E7 monoclonal antibody and complement of CHO-K1 □ and J1 o. In contrast to the CHO-K1 parental cell, J1 was readily killed. The percentage survival is relative to a complement control.

Antigen Assay. Cells were plated in the presence of complement (1.5% normal rabbit serum) in increasing concentrations of antiserum or hybridoma supernatant; 6 days later, the plates were fixed and stained, the number of colonies determined, and survival curves plotted. The difference between cells scored as killed or not killed is illustrated in Fig. 1.

Karyotypic and Isozyme Analysis of Hybrids. Human chromosome content of hybrids was determined cytogenetically by trypsin banding and Giemsa-11 staining (8, 16) and by analyzing isozymes specific for each human chromosome as previously described (17).

Glycolipid Conversion. The macroglycolipid (MGL) fraction of human red blood cell membranes was prepared by the method of Dejter-Juszynski et al. (10, 18). CHO-K1 cells were incubated with a solution of MGL (1 mg/ml) in PBS for 30 min. Cells were then washed and tested with monoclonal antibody using the standard cytotoxicity assay. Control cells were incubated for 30 min in PBS.

RESULTS

Chromosomal Assignment. Survival curves showing the behavior of J1, a human–Chinese hamster hybrid containing chromosome 11 as its only human chromosome (8), and CHO-K1, the Chinese hamster parental cell line, when

Table 1. Segregation Analysis of Antigen Recognized by E7 Monoclonal Antibody

Hybrid	Human chromosome present	Killing action displayed by E7 monoclonal antibody
822-19B	1,2,3,5,6,10,*11*,14,15,19,20,21,22	Killed
750-7	4,9,*11*,16,X	Killed
68-11	1,5,6,8,9,*11*,12,14,15,19,20,21,22,X	Killed
68-14	4,5,6,7,8,9,*11*,12,14,15,18,19,20,21,22	Killed
72-4B	3,8,10,*11*,12,17,21	Killed
822-5a	2,9,*11*,12,13,19,21,22	Killed
68-12	2,3,4,5,6,7,9,*11*,12,15,16,18,19,20,21,22	Killed
822-59b	4,7,14	Not killed
68-10A	4,8,12,14,15,18,19,21,22,X	Not killed
68-16	5,6,8,21,22	Not killed

tested with the E7 monoclonal antibody are shown in Fig. 1. No complement-mediated cytotoxicity is demonstrable for CHO-K1 at concentrations of monoclonal antibody 1000 times greater than that which is completely lethal to J1. This provides evidence that this antigen activity is dependent on the presence of human chromosome 11. To confirm this, a panel of independently isolated human–Chinese hamster hybrids which contain a combination of human chromosomes was tested with E7 monoclonal antibody. As shown in Table 1, there was 100% concordance of this activity with the presence of chromosome 11.

Regional Mapping. We have previously isolated and characterized a regional clone panel from J1 containing different deletions of chromosome 11 (9, 19). Members of this panel were tested with E7, and the results are shown in Table 2, which indicate the genetic component responsible for E7 activity resides on the short arm of 11 (11p13 → 11pter).

Mutant Analysis. Because we have previously identified human antigens a_1 and a_3 with the same map location (9), we examined a mutant which was a_1^- a_3^+ and contained a normal-appearing chromosome 11 (10). This mu-

Table 2. Characteristics of Hybrids and Human Cells Tested with E7 Monoclonal Antibody

| Cell	Deletions in chromosome 11	Antigenic phenotype	Killing action displayed by survival curve analysis to E7 monoclonal antibody
J1	None	$a_1^+a_2^+a_3^+$	Killed
J1-7	p11 → pter	$a_1^-a_2^+a_3^-$	Not killed
J1-11	q13 → qter	$a_1^+a_2^-a_3^+$	Killed
J1-23	p13 → pter	$a_1^-a_2^+a_3^-$	Not killed
J1-35	None	$a_1^-a_2^+a_3^+$	Not killed
HeLa		$a_1^+a_2^+a_3^+$	Killed
Altered HeLa		$a_1^-a_2^+a_3^+$	Not killed

tant clone showed no cytotoxicity when tested with the monoclonal antibody (Table 2). This suggests that E7 may recognize the a_1 antigen but not a_3.

We have previously isolated and described a variant clone of HeLa which differs in morphology from the standard S3-HeLa (20). The altered HeLa has a round morphology and attaches poorly to plastic. Figure 2 compares the morphology of the altered HeLa with that of standard HeLa. We have previously shown that altered HeLa does not express the a_1 antigen by its failure to adsorb a_1 activity from a_1^+ polyclonal antisera. It is also unable to elicit anti-a_1 antibodies in rabbits in contrast to standard HeLa. Altered HeLa does express a_2 and a_3, two other chromosome 11 antigen-associated activities. The monoclonal E7 was, therefore, tested with these two HeLa cell lines, and it was found that standard HeLa was killed and altered HeLa was not (Table 2). Mutant analysis, therefore, shows that a_1 and the antigen recognized by E7 map on the same region of chromosome 11 and variants of both J1 and HeLa which have lost the a_1 antigen have lost the antigen recognized by E7.

Glycolipid Conversion. We have previously shown that a_1 antigenic activity is found in the macroglycolipid (MGL) fraction of the human erythrocyte membranes (10). Other workers have shown that it is possible to convert cholera toxin-resistant cells to cholera toxin sensitivity using the glycolipid GM_1 ganglioside (21, 22). Lymphocytes have been made reactive to a teratoma-specific antiserum by treating them with organic extracts of teratoma cells (23). We therefore tested the ability of the MGL fraction to convert CHO-K1 cells to antigen positive. As was shown previously (Fig. 1), CHO-K1 cells are not killed by E7 at high concentrations but, as shown in Fig. 3, MGL treatment produced cells which were killed by the monoclonal antibody. This provides evidence that the MGL fraction which contained a_1 activity also has activity for E7. We therefore conclude that E7 recognized the same or a similar antigenic activity as that previously identified as a_1.

DISCUSSION

The availability of the E7 monoclonal antibody which recognizes the a_1 cell-surface antigenic determinant will allow further elucidation of the structure of this molecule. Monoclonal antibodies which are specific for single antigenic determinants will be particularly useful when studying complex structures like macroglycolipids. The E7 monoclonal antibody belongs to the IgM class and is extremely efficient in complement-mediated cytotoxicity tests, which makes it useful for the types of genetic analyses we use to study cell-surface molecules. It will also be useful for establishing the cellular and tissue distribution of this antigen. To date, the only human cell line identified which lacks this antigen is the altered HeLa clone, but more cell lines are
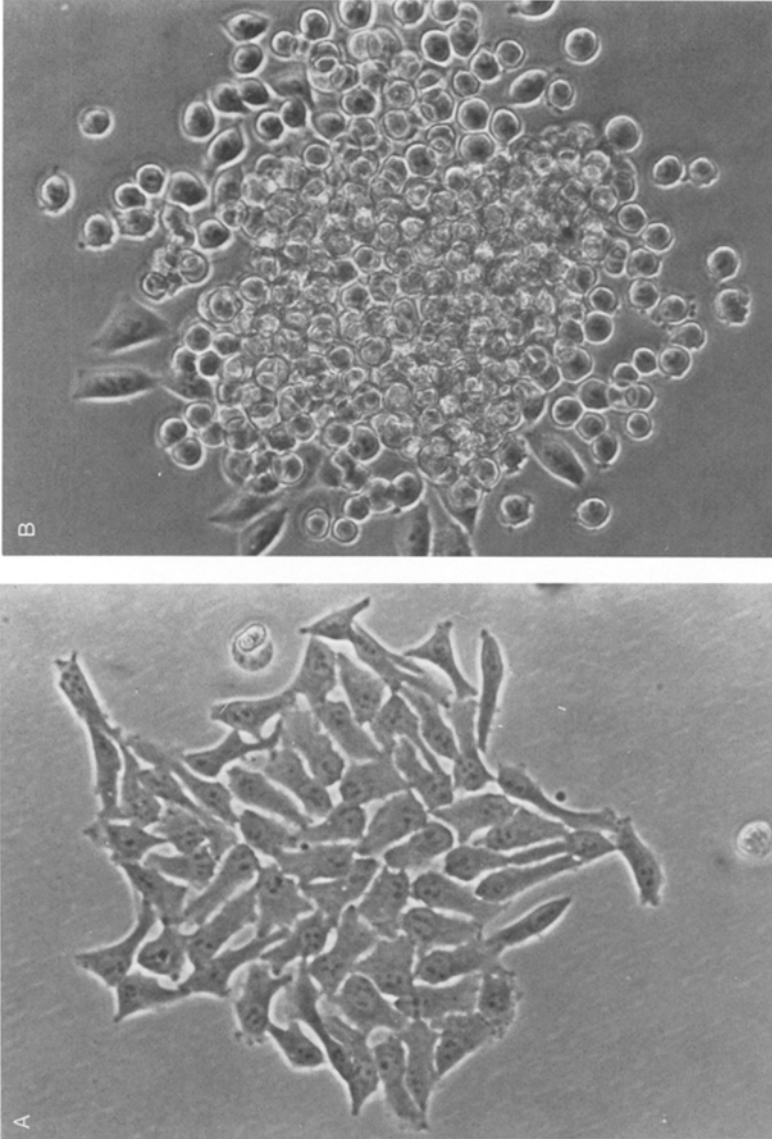
Fig. 2. Morphology of standard HeLa (A) and altered HeLa (B) (magnification ×120; reduced 35% for reproduction).
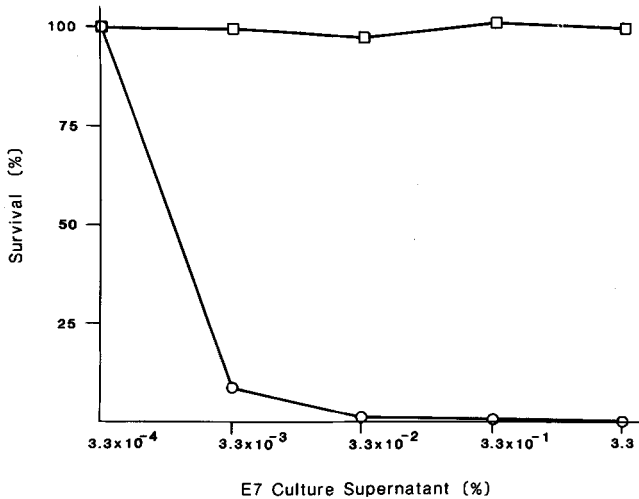
Fig. 3. Percentage survival in E7 monoclonal antibody and complement of control CHO-K1 □ and CHO-K1 cells converted with the macroglycolipid fraction (MGL) of human red blood cell membranes ○. Treatment of CHO-K1 with MGL converted this cell from one which was resistant to the action of E7 to one that was readily killed.

being tested and lines with significant quantitative differences have been identified.

A number of cell-surface molecules have been assigned to human chromosome 11. The relationship of these antigens to the antigens we have called a_1, a_2, and a_3 is not firmly established. Indeed we have genetic evidence that the activity we call a_2, which is encoded for by the long arm of 11, consists of at least three separate activities. Barnstable et al. (5) have described monoclonal antibody which recognizes an antigen encoded for by the short arm of 11 which appears to be a glycolipid. This monoclonal antibody, W6-34, also appears to recognize the antigen we call a_1 (24). This was determined using a similar experimental approach to that described for characterization of the E7 monoclonal antibody. We have also shown that the 105,000-dalton glycoprotein which is recognized by monoclonal antibody F10.44.2, assigned to chromosome 11 by Goodfellow et al. (6), maps on the same region of chromosome 11 as the a_3 antigen (24). Messer Peters et al. (7) have recently assigned an 85,000-dalton glycoprotein to chromosome 11 using a monoclonal antibody 4F2, which appears different from those previously described.

The availability of these different monoclonal antibodies recognizing cell-surface antigens encoded for by human chromosome 11 should facilitate further manipulation and study of this human chromosome.

ACKNOWLEDGMENTS

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