DETECTION OF CELL SURFACE AND INTRACELLULAR ANTIGENS BY HUMAN MONOCLONAL ANTIBODIES

Hybrid Cell Lines Derived from Lymphocytes of Patients with Malignant Melanoma*

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Defining the antigenic changes that accompany malignant transformation and detecting whether these changes elicit immune recognition in the host of origin are central concerns of tumor immunology. Serological approaches to these issues have been vastly strengthened with the advent of hybridoma technology (1). Monoclonal antibodies are providing much new information about the antigenic structure of experimental and human cancers, and hybridoma analysis promises to be of great value in dissecting the humoral immune response to tumor antigens in tumor-bearing animals and humans.

The problem with past efforts to resolve the question of tumor-related immune response in humans using conventional serology has had to do with the issue of specificity (2). With the exception of virus-related antigens on tumors such as Burkitt's lymphoma and hepatoma (3), and HLA antigens and blood group antigens, the nature and significance of other classes of human cancer antigens detected by human antibody are unknown. To assess the frequency and specificity of antibodies reacting with surface antigens of human cancer cells, we have analyzed the autologous reactivity of sera from a series of patients with melanoma, astrocytoma, renal cancer, and leukemia (4-10). Three classes of antigens detected by autologous antibody have been defined in this way. Class 1 antigens are restricted to autologous tumor cells, not being detected on any other cell type, normal or malignant. Class 2 antigens are shared antigens, found on a proportion of allogeneic tumors as well as on autologous tumors; recent evidence indicates that some class 2 antigens are autoantigenic differentiation antigens, since they are detected on a restricted range of normal tissues (11). Class 3 antigens are widely distributed on normal and malignant cultures; these broadly represented antigens have not been extensively analyzed. Whereas class 3 reactivity is relatively common, antibodies to class 1 and class 2 antigens are found infrequently (~10% of patients).

To extend these studies of humoral immune reactions of cancer patients, we have been exploring techniques for the production of human monoclonal anti-

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bodies using hybridoma methodology. Our initial experience with immunoglobulin-secreting clones derived from lymphocytes of patients with malignant melanoma is reported here.

Materials and Methods

Myeloma/Lymphoblastoid Cell Lines. The human myeloma cell line SKO-007 (12) was obtained from Beckton, Dickinson & Co., Sunnyvale, CA, and was rendered free of mycoplasma contamination by Dr. J. Fogh, Sloan-Kettering Institute. SKO-007 secretes ε heavy chain and λ light chain. The human LICR-LON-HMy2 (LICR-2) cell line (13) was obtained from Drs. M. O'Hare, P. Edwards, and M. Neville, London Branch of the Ludwig Institute for Cancer Research. This lymphoblastoid cell line secretes γ1 heavy chain and κ light chain and expresses Epstein-Barr virus (EBV) nuclear antigen (EBNA). The human lymphoblastoid cell line GM 4672, developed from the GM 1500 cell line by Croce et al. (14), was obtained from the Human Genetic Mutant Cell Repository, Institute of Medical Research, Camden, NJ. This cell line secretes γ1 heavy chain and κ light chain.

The mouse myeloma cell line NS-1 (15) was obtained from Dr. U. Hämmerling, Sloan-Kettering Institute. These cell lines were grown in RPMI 1640 medium (containing 2 mM glutamine, 1% nonessential amino acids, 100 U/ml penicillin, and 1 µg/ml streptomycin), 7.5% (vol/vol) fetal bovine serum (FBS), and 20 µg/ml 8-azaguanine. No growth occurred in medium containing 4 × 10⁻⁷ M aminopterin.

Fusion Procedure. Lymph node and tumor specimens from 33 patients with malignant melanoma were minced with fine scissors under sterile conditions. The resulting cell suspension was washed twice in RPMI 1640 medium and used as the source of lymphocytes for fusion. Using lymph node and tumor-infiltrating lymphocytes, 36 fusions were performed with LICR-2, 24 fusions with SKO-007, 8 with GM 4672, and 11 with NS-1. Peripheral blood lymphocytes from 25 melanoma patients were purified from heparinized venous blood by Ficoll-Hypaque (Pharmacia Fine Chemicals, Division of Pharmacia Inc., Piscataway, N.J) gradient centrifugation and 41 fusions performed with LICR-2, 32 with SKO-007, and 6 with NS-1. For further enrichment of B cells, T lymphocytes were removed by rosetting with neuraminidase-treated sheep erythrocytes and centrifugation through a Ficoll-Hypaque gradient.

Lymphocytes and myeloma/lymphoblastoid cells were fused at ratios of 1:1 or 2:1 for 3 min at 37°C in 0.2 ml 41.5% (wt/vol) polyethylene glycol (mol wt 4,000; J. T. Baker Chemical Co., Phillipsburg, N.J) dissolved in 15% (vol/vol) dimethyl sulfoxide. Between 2 × 10⁶ and 5 × 10⁷ lymphocytes were used in each fusion experiment. After fusion, cells were washed and left overnight in RPMI 1640 medium containing 15% FBS. The cells were then resuspended in RPMI 1640 medium containing 15% FBS, 2 × 10⁻⁴ M hypoxanthine, 4 × 10⁻⁷ M aminopterin, 3.2 × 10⁻⁵ M thymidine, and 2 × 10⁻⁵ M 2-mercaptoethanol (HAT medium) and plated at 10⁵ cells per well into Costar 3696 96-well plates (Costar, Cambridge, MA), preseeded with feeder layers prepared from BALB/c or C57BL/6 splenocytes (10⁵ cells/well) or peritoneal cells (1–2 × 10⁶ cells/well). Cells were maintained in HAT medium for a minimum of 4 wk. Growing clones were defined as wells with continuing outgrowth more than 2 wk after fusion. Growing clones derived from fusions with LICR-2, SKO-007, and GM 4672 appeared between 3 and 8 wk after fusion, while growing clones from fusions with NS-1 appeared between 2 and 6 wk. To compare the results of fusions with different sources and numbers of lymphocytes and different myeloma/lymphoblastoid cell lines, the fusion frequency was normalized and expressed as the median number of wells with growing clones per 10⁷ lymphocytes fused.

Immunoglobulin Assays. Supernatants from wells containing growing clones were assayed for human μ, γ, or α heavy chains by an enzyme-linked immunosassay. Costar 3696 96-well plates were precoated with human IgG (50 µg/ml), IgA (50 µg/ml), or IgM (10

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Abbreviations used in this paper: EBNA, Epstein-Barr virus nuclear antigen; EBV, Epstein-Barr virus; FBS, fetal bovine serum; FITC, fluorescein isothiocyanate; HAT, hypoxanthine-aminopterin-thymidine; IA, immune adherence; PA, protein A; PBS, phosphate-buffered saline.
μg/ml) (Cappel Laboratories, Cochranville, PA) overnight at 4°C. The precoated wells were washed with phosphate-buffered saline (PBS) pH 7.5 and incubated with gamma globulin-free FBS (Gibco Laboratories, Grand Island, NY) for 30 min. Goat anti-human μ, γ, or α heavy chain antibodies linked to alkaline phosphatase (Sigma Chemical Co., St. Louis, MO), diluted 1:100, were mixed (1:1 vol/vol) with test supernatants or with IgG, IgA, or IgM standards diluted in RPMI 1640 medium containing 15% FBS (final concentrations of standards were 10 ng/ml, 100 ng/ml, 1 μg/ml, 10 μg/ml, and 100 μg/ml) and incubated for 90 min. The mixtures were then transferred to precoated wells, incubated for 60 min, and washed with PBS. Alkaline phosphatase activity was detected using a p-nitrophenyldisodium phosphate substrate (Sigma Chemical Co.), with changes in optical density measured on an Artek Model 210 Reader (Artek Systems Corp., Farmingdale, NY). The assay detected <100 ng/ml of μ, γ, or α heavy chains and was specific for each Ig class over a range of <100 ng/ml to >100 μg/ml. The LICR-2 and GM 4672 parental lines produced 10-100 ng/ml and 10-500 ng/ml γ chain, respectively. Neither μ, γ, or α was detected in supernatants of SKO-007 or NS-1.

Antibody Reactivity to Cell Surface and Intracellular Antigens. Supernatants from wells containing ≥200 ng/ml Ig were screened for reactivity to cellular antigens using the following panel of 20 human tumor cell lines: melanomas (SK-MEL-13, 19, 23, 28, 29, 37, 93, 147, 165, and MeWo); malignant gliomas (U251 MG, SK-MG-3); epithelial cancers (SK-RC-7, SK-RC-9, BT-20, CAMA, 253J, HT29, OV2774, and Calu-1). To detect cell surface antigens, target cells were plated in Falcon 3034 plates (Falcon Labware, Oxnard, CA), and erythrocyte-rosetting assays for IgG (protein A [PA] assay) and IgM (immune adherence [IA] assay) were performed as previously described (5, 8). IgA antibodies were detected by indicator cells prepared by conjugating purified anti-human IgA (Accurate Chemical & Scientific Corp., Westbury, NY) to human erythrocytes with 0.01% chromium chloride. Absorption tests were performed according to previously described procedures (4). To detect intracellular antigens, supernatants were screened by indirect immunofluorescence tests using the same panel (see above). Target cells growing in Falcon 3034 plates were fixed with a 1:1 (vol/vol) methanol/acetone mixture for 5 min at room temperature. The cells were incubated with supernatant for 1 h at room temperature, washed, and incubated with a 1:50 dilution of goat anti-human Ig conjugated to fluorescein isothiocyanate (FITC) (Cappel Laboratories) for 45 min. Fluorescence was evaluated with a Leitz Dialux 20 microscope (E. Leitz, Inc., Rockleigh NJ).

Chloroform-Methanol Extraction. Cells were extracted with chloroform/methanol by previously described procedures (16). Antibody inhibition tests were carried out by mixing the cell extract with antibody containing supernatant (diluted two dilutions below the endpoint), incubating for 1 h at 20°C, and testing for residual antibody reactivity using SK-RC-9 target cells.

Results

Cell Growth After Fusion of Lymphocytes with Myeloma/Lymphoblastoid Cell Lines: Fusion Frequency, Immunoglobulin Production, and Clonal Stability. 158 fusions were performed with lymphocytes from lymph node, tumor infiltrate, and peripheral blood of 52 melanoma patients. Wells containing clonal outgrowth appeared most frequently after fusion with the NS-1 mouse myeloma line (25 clones per 10^7 lymphocytes fused) and the frequency of clonal outgrowth was similar with lymphocytes from lymph node and peripheral blood. From 3 to >25 times lower frequency of growing clones was obtained after fusion with the three human myeloma/lymphoblastoid cell lines. In the case of lymphocytes from lymph node, fusions with LICR-2 resulted in a higher frequency of clonal outgrowth (eight clones per 10^7 lymphocytes fused) than fusions with SKO-007

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2 Detailed results concerning fusion frequency and immunoglobulin production in relation to myeloma/lymphoblastoid cell lines and source of lymphocytes are available upon request.
or GM 4672 (one clone per $10^7$ lymphocytes fused). Uniformly poor results were obtained in LICR-2 or SKO-007 fusions with peripheral blood as the source of lymphocytes (<1 clone per $10^7$ lymphocytes fused). Enriching the B cell population of peripheral blood lymphocytes by depleting T cells before fusion with LICR-2 resulted in a 5 to >20 times higher frequency of growing clones.

Immunoglobulin heavy chains $\mu$, $\gamma$, or $\alpha$ were detected in 50–80% of wells containing growing clones. The levels of Ig production ranged from 0.3 to 40 $\mu$g/ml and the relative proportion of $\mu$-, $\gamma$-, and $\alpha$-positive wells varied from specimen to specimen. No apparent relation was found between level and/or class of Ig production and the different fusion partners or sources of lymphocytes.

The stability of Ig production by cells derived from fusions with NS-1 and LICR-2 was examined by subculturing Ig$^+$ wells using a limiting dilution technique. Cells from 77% (76/99) of Ig$^+$ wells from LICR-2 fusions continued to produce Ig after one subculture (between 2 and 3 mo after fusion), and 61% remained Ig$^+$ after a second subculture (at 3–4 mo). A lower percentage of mouse/human clones had persistent Ig production; 58% (43/74) of Ig$^+$ wells from NS-1 fusions remained Ig$^+$ after the first subculture (at 2 mo after fusion), and 30% were Ig$^+$ after the second subculture (at 3 mo).

**Immunoglobulin Reactivity with Cell Surface Antigens: Definition of the Ma4 Antigen.** Supernatants of Ig$^+$ wells were tested for reactivity with cell surface antigens by erythrocyte-rosetting assays using a panel of 20 cell lines, including 10 melanomas, 2 gliomas, and 8 epithelial cancers (see Materials and Methods). Of 771 wells screened, positive reactions were observed with supernatants from 6 wells (0.8%). Efforts to isolate antibody-secreting clones from these 6 wells resulted in the establishment of a cell line, designated Ma4, that continued to produce an IgM antibody to a surface antigen of human cells. The Ma4 line was derived from a fusion of LICR-2 with lymphocytes from a regional lymph node of a 35-yr-old man with recurrent malignant melanoma. The line has been subcloned (1 cell/well) four times and has maintained stable production of IgM (5 $\mu$g/ml) and IgG (2 $\mu$g/ml) over a 12-mo period. The Ma4 cell line is tetraploid by flow cytometry and contains only human chromosomes by karyotypic analysis. Analysis by sodium dodecyl sulfate/polyacrylamide gel electrophoresis shows that the Ma4 line secretes two distinct heavy chains, $\mu$ and $\gamma$, and light chains, $\kappa$ and $\lambda$.

Supernatants from cultures of the Ma4 cell line were found to be highly reactive with SK-RC-9 (an established cell line derived from human renal cancer) (Fig. 1). Reactivity was detected by 1A assays for IgM antibody, but not by assays detecting IgA or IgG antibodies. (Serum from the patient providing lymphocytes used in the construction of the Ma4 cell line was unreactive [titer <1:2] with SK-RC-9 target cells by 1A assay). Fig. 2 illustrates 1A tests with Ma4 supernatant on four other established human cell lines; reactions were seen with SK-LC-13, a cell line derived from lung cancer, and AlAb, a cell line derived from breast cancer, but not with two melanoma cell lines. Fig. 3 demonstrates analysis of Ma4 antibody reactivity by absorption tests and Table I summarizes the results of direct tests and absorption analysis using a panel of 81 different cell types.

The antigen detected by Ma4 antibody was found to be heat stable (100°C for 10 min) and resistant to treatment with trypsin and proteinase K. Chloroform/
methanol extracts of the Ma4-positive cell line SK-RC-9 completely inhibited Ma4 antibody reactivity. Extracts from Ma4-negative cell lines (253J and BT-20) had no inhibitory activity.

Screening of Immunoglobulin for Reactivity to Intracellular Antigens. Supernatants
### Table 1

Results of Direct Tests and Absorption Analysis of IA Reactivity of Ma4 Culture Supernatant: Definition of the Ma4 Antigen System

| Cell line | Titer* | Absorption² | Cell line | Titer* | Absorption² | Cell line | Titer* | Absorption² |
|-----------|--------|-------------|-----------|--------|-------------|-----------|--------|-------------|
| Renal cancer |        |             | Colon cancer |        |             | SK-MG-9 |        |             |
| SK-RC-4 |        |             | HT-29 |        | +           | SK-MG-10 |        |             |
| SK-RC-7 |        |             | SW-620 |        |             | SK-MG-13 |        |             |
| SK-RC-9 | 1:1280 | +           | SW-480 | 1:2 | +           | U751 MG |        |             |
| SK-RC-28 |        | +           | SW-1222 |        | +           | U753 MG | 1:16 | +           |
| SK-RC-38 |        |             | Melanoma |        |             | T98 |        |             |
| Bladder cancer |        |             | SK-MEL-13 (AH) |        |             | Hematopoietic cells |        |             |
| T-24 | 1:64 | +           | SK-MEL-19 (AL) |        |             | SK-LY-16 |        |             |
| 639v |        |             | SK-MEL-21 (AN) |        |             | SK-LY-18 |        |             |
| Scb |        |             | SK-MEL-23 (AP) |        | +           |              |        |             |
| RT-4 |        |             | SK-MEL-26 (AS) |        |             | EBV-transformed B cells |        |             |
| 253j |        |             | Melo |        |             | AH |        |             |
| 647v |        |             | SK-MEL-28 (AU) |        |             | AV |        |             |
| TCC-SUP |        |             | SK-MEL-29 (AV) |        | +           | AZ |        |             |
| Breast cancer |        |             | SK-MEL-30 (AW) |        |             | DX |        |             |
| MCF-7 |        |             | SK-MEL-51 (AX) |        |             |              |        |             |
| CAMA |        | +           | SK-MEL-33 (AZ) | 1:128 | +           | EG |        |             |
| BT-20 |        |             | SK-MEL-37 (BD) |        |             |              |        |             |
| AIAb | 1:128 | +           | SK-MEL-41 (BH) |        |             |              |        |             |
| Lung cancer |        |             | SK-MEL-50 (BO) |        |             | Adult fibroblasts |        |             |
| Calu-1 |        |             | SK-MEL-90 (DO) |        |             | AS |        |             |
| SK-LC-6 | 1:4 | +           | SK-MEL-95 (DX-1) |        |             | BG |        |             |
| SK-LC-8 |        |             | SK-MEL-93 (DX-2) |        |             | DX |        |             |
| SK-LC-13 | 1:4000 | +           | SK-MEL-110 (EF) | 1:52 | +           | RC-9 |        |             |
| Ovarian, cervical and uterine cancer |        |             | SK-MEL-118 (EQ) |        |             | Fetal cell lines |        |             |
| ME-180 |        |             | SK-MEL-127 (EZ) |        |             | WI-38 |        |             |
| OV-2774 | 1:256 | +           | SK-MEL-131 (FD) |        |             | F-3 Brain |        |             |
| SK-OV-5 |        |             | SK-MEL-147 (FF) |        | +           | Flow 5000 |        |             |
| Colo 516 |        |             | SK-MEL-158 (GL) |        |             |              |        |             |
| ROAC |        |             | Malignant glioma |        |             | Normal kidney |        |             |
| SK-UT-1 |        |             | SK-MG-1 | 1:2 | +           | NK-1 |        |             |
|                               |        |             | SK-MG-3 |        |             | NK-2 |        |             |

* (--) indicates no reaction in direct tests of undiluted culture supernatants.

² Culture supernatant (diluted from 1:8 to 1:256 according to endpoint) was absorbed with the indicated cell type and tested for residual activity with SK-RC-9 renal cancer target cells.
from the 771 Ig* wells that were tested for reactivity to cell surface antigens (see above) were also tested for reactivity to intracellular antigens by indirect immunofluorescence assays. 27 (3.5%) wells contained antibodies reacting with nuclei, nucleoli, cytoskeletal elements, Golgi complex, and other cytoplasmic components. Cell lines from five of these wells have maintained stable antibody production over a 5-8 mo observation period. All five cell lines produce IgM antibody and were derived (by limiting dilutions) from fusions of lymph node lymphocytes. Antibodies M307 and M311 came from fusions with SKO-007; antibodies M304 and M305 from fusions with LICR-2; and antibody M54 from a fusion with NS-1. Antibody M311 showed granular staining of the nucleus (Fig. 4A). Antibodies M305 and M307 reacted with cytoplasmic components in a wide range of cultured cell types; M307 stained a cytoskeletal network (Fig. 4B) and staining with M305 showed a dense reticular pattern (Fig. 4C). Antibodies M304 and M54 also reacted with cytoplasmic components, but in the case of these antibodies reactions were restricted to certain cell types. Antibody M304 reacted with cells of neuroectodermal origin, including astrocytomas (9 of 9 tested), melanomas (9 of 16 tested), neuroblastomas (1 of 3 tested), and normal melanocytes (Figs. 4D and 5A). No reactions were observed with epithelial cancers or with cultures of normal kidney or fibroblasts. On the other hand, antibody M54 reacted primarily with epithelial cell lines, including breast cancers (four of five tested) and normal kidney epithelium (four of five tested) (Fig. 5B). Antibody M54 did not react with any cell line of neuroectodermal origin.

Discussion

In contrast to the success of hybridoma technology in the production of mouse and rat monoclonal antibodies, comparable studies with human antibody-producing hybrids have lagged behind. The general experience of many investigators has been that fusion with drug-marked human myeloma or lymphoblastoid cell lines resulted in few clones with growth potential. Fusion of human lymphocytes with mouse myeloma resulted in larger numbers of growing clones, but these appeared to be unstable with regard to Ig production. Recent studies (12–14, 18–23) and the results reported here indicate that methods for the construction of Ig-secreting human/human hybrids or mouse/human hybrids have been considerably improved and are now at the stage that a fine analysis of the humoral immune response to cancer in humans can begin. Nonetheless, a number of technical questions remain to be clarified. Although Ig-secreting clones were obtained from fusions with the myeloma and lymphoblastoid lines used in this study and in the parallel study of Cote et al. (23), there were significant differences in the frequency of clonal outgrowth with each of the lines. In the case of the human lines, LICR-2 was clearly the superior partner. As the LICR-2 line is EBNA+, and therefore presumably a source of transforming EBV, the role of EBV in the better performance of LICR-2 needs to be analyzed. Although there is no doubt that true hybrids can be obtained from LICR-2 fusions, as shown in this report and by Edwards et al. (13), Sikora et al. (20), and Cote et al. (23), it remains to be determined what proportion of clones growing in Ig* wells are human/human hybrids and what proportion are EBV-transformed
Intracellular antigens detected by IgM antibodies in supernatants of cultures derived from fusions of lymphocytes from lymph nodes of melanoma patients with LICR-2 (M304 and M305 antibodies) and SKO-007 (M307 and M311 antibodies). (A) M311 detects a nuclear antigen (target SK-MEL-63 melanoma cell line); x 1,000; (B) M307 detects a cytoskeletal structure (target cell W1-38 fetal fibroblasts); x 400; (C) M305 detects a dense cytoplasmic network (target cell W1-38); x 200; (D) M304 detects a cytoplasmic antigen expressed by cells of neuroectodermal origin (target cell SK-MEL-93 melanoma cell line); x 400.

lymphoblasts. Another problem is the lower frequency of clonal outgrowth after fusion of LICR-2 with peripheral blood lymphocytes. As peripheral blood is the most readily available source of lymphocytes from patients, this low yield of Ig-secreting clones will be a limiting factor in the application of LICR-2. There are several explanations for this observation, including (a) the low percentage of B cells in peripheral blood, (b) the possibility that the differentiation stage of B cells in the peripheral blood is not optimal for hybrid formation or hybrid stability or (c) destruction of hybrid cells by cytotoxic T cells elicited by surface
Figure 5. Reactivity of M304 antibody (A) and M54 antibody (B) with a panel of cultured cells using indirect immunofluorescence assays. Horizontal bars indicate intensity (0 to 3+) of immunofluorescence reactions. (*) In the case of 833K teratocarcinoma cell line, only 10% of cultured cells reacted with M54 antibody.
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antigens contributed by the LICR-2 partner. If the latter possibility is involved in the low frequency of clonal outgrowth after LICR-2 fusion with peripheral blood lymphocytes, removal of T cells before fusion should increase the frequency of Ig-secreting clones. The outcome of initial experiments indicates that this may be the case. Comparing results of fusion with the human myeloma/lymphoblastoid cell lines to fusion with the NS-1 mouse myeloma showed that in each instance, including fusions with peripheral blood lymphocytes, the frequency of outgrowth of NS-1-derived clones was substantially higher. In addition, mouse/human clones generally subcloned with higher efficiency than the human/human clones. However, Ig production by the mouse/human clones was less stable than the human clones. In a parallel but independent study from this laboratory (23), mouse/human clones were found to be as stable as human/human clones and mouse/mouse clones with respect to Ig production. The reason for this discrepancy between the two studies is being analyzed.

Reactivity of Ig-containing supernatants with intracellular antigens was found to be significantly higher than reactivity with cell surface antigens. This finding could have several explanations, including (a) greater polymorphism of cell surface antigens vs. intracellular antigens, requiring a larger variety of cell lines and cell types in the screening panel to identify antibodies reacting with cell surface antigens; (b) greater range of antigenic determinants within the cell than on the cell surface; (c) loss or low expression of certain cell surface antigens on cells in vitro vs. cells in vivo; and (d) immunological tolerance that restricts autoantibodies to cell surface antigens to a greater degree than to intracellular antigens. To pursue these points, it will be important to use noncultured cells as serological targets, to determine whether in vitro sensitization to surface antigens before fusion increases the frequency of surface antibodies, and to analyze the results of fusions with lymphocytes from patients with high-titered antibody against known cell surface antigens, e.g., HLA or blood group antigens.

The cell surface reactivity seen in our initial screening has not conformed to any known antigenic system or differentiation pathway. For example, specificity testing of the antibody produced by the Ma4 clone has not shown any recognizable reactivity pattern. The IgM antibody secreted by the Ma4 line reacted with 19 of the 61 tumor cell lines tested, with no predilection for any particular cell type. Similarly, the IgG antibody produced by a stable mouse/human hybrid (Ri37) derived from the lymphocytes of a patient with breast cancer and reacting with a different set of tumor cell lines showed no differentiation-related pattern (23). In contrast, Irie et al. (24) have analyzed a human antibody produced by EBV-transformed lymphocytes from a patient with melanoma that reacted with an antigen expressed by cells of neuroectodermal origin. Systematic analysis of a large number of cell surface-reactive human monoclonal antibodies, using both autologous as well as allogeneic normal and malignant cells as targets, should give insight into the nature of autoantigenic surface antigens and determine whether any have the characteristics of tumor-specific antigens. Similarly, the serological dissection of intracellular structures by human monoclonal antibodies
permits a new level of precision in the study of autoimmune recognition of normal and malignant cells.

Summary

This study represents an initial attempt to analyze the humoral immune reactions of patients with malignant melanoma by hybridoma methodology. Using lymphocytes from regional lymph nodes, peripheral blood and tumor infiltrates, 158 fusions were performed with SKO-007 (human myeloma line), LICR-LON-HMy2 (LICR-2), GM 4672 (human lymphoblastoid lines), or NS-1 (mouse myeloma line). Fusion of lymph node lymphocytes with NS-1 resulted in a 3-4 times higher frequency of clones than fusion with LICR-2, and a 10 times higher frequency than fusion with SKO-007 or GM 4672. In the case of peripheral blood lymphocytes, fusion with NS-1 gave >25 times higher frequency of clones than fusion with LICR-2 or SKO-007. Production of human \(\mu, \gamma,\) or \(\alpha\) heavy chains was detected in 50-80% of wells containing growing clones, and the levels of immunoglobulin ranged from 0.3 \(\mu\)g to 40 \(\mu\)g/ml. NS-1-derived clones could be easily subcultured, while LICR-2 and SKO-007 clones grew more slowly on subculturing. In this study, Ig secretion appeared to be a more stable property of LICR-2-derived clones than NS-1-derived clones. A panel of 20 human cancer cell lines was used to screen 771 Ig-secreting cultures for antibody to cell surface or intracellular antigens. Reactivity with cell surface antigens was found infrequently (6 cultures), whereas reactivity with intracellular antigens was more common (27 cultures). A new cell surface antigen with properties of a glycolipid was defined with an IgM monoclonal antibody secreted by a tetraploid cell derived from a fusion of LICR-2 with lymphocytes from the axillary lymph node of a patient with melanoma. The hybrid cell line has been subcloned four times and secretes 5 \(\mu\)g IgM/ml. The antigen detected by this IgM antibody was found on 5 of 23 melanoma cell lines and 12 of 30 epithelial cancer cell lines. No reactions were found with 11 cultures derived from normal cells. Stable cell lines secreting human antibody that detected nuclei, nucleoli, cytoskeletal elements, Golgi complex, or other cytoplasmic components were also isolated in this study. One of these antibodies detected an intracellular antigen that is restricted to cells of neuroectodermal derivation, and a second antibody reacted primarily with cells of epithelial origin. Using these methods to isolate and analyze human monoclonal antibody, it should now be possible to define the repertoire of the humoral immune response to melanoma.

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