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Immunology

Generation of human monoclonal antibodies reactive with cellular antigens

(hybridoma/cell-surface antigens/intracellular antigens/cancer immunology)

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ABSTRACT **Human lymphocytes from lymph node, peripheral blood, spleen, and tumor specimens have been fused with the LICR-LON-HMy2 (LICR-2) or SKO-007 human cell lines or the NS-1 mouse myeloma line. Over 75 fusions with the three myeloma–lymphoblastoid lines have been performed. Several factors appeared to improve the fusion outcome, including maintenance of the myeloma–lymphoblastoid lines in logarithmic phase growth at ≥95% viability, a delay of 24 hr in the introduction of aminopterin to the fused cells, and preselection of the fetal calf serum used in the medium. For a given number of lymphocytes, fusions with NS-1 produced 5–20 times more clones than fusions with LICR-2 or SKO-007, and LICR-2 produced 4 times as many clones as SKO-007. The percentage of clones secreting human immunoglobulin, the range of immunoglobulin production, and the proportion of IgM, IgA, and IgG secretors were comparable for clones derived from the three myeloma–lymphoblastoid lines. Stable Ig-secreting clones were isolated with approximately equal frequency from LICR-2 and NS-1 fusions. A number of stable clones producing human monoclonal antibodies reacting with cell-surface, cytoplasmic, or nuclear antigens have been isolated from tumor-bearing patients and normal individuals. A surface antigenic system present on normal and malignant cells has been defined with a human monoclonal antibody derived from a patient with breast cancer. Techniques for producing human monoclonal antibody now appear to be sufficiently advanced to initiate a serological dissection of the humoral immune response to cancer.**

The serological analysis of human cancer has been revolutionized by the introduction of the hybridoma technology (1). Knowledge about the surface antigenic structure of several types of human cancers has advanced rapidly with mouse monoclonal antibodies as serological probes, and application of these reagents to cancer diagnosis and therapy is underway. However, production of human monoclonal antibodies has proved more difficult to achieve. Despite much effort by many laboratories around the world, there are relatively few reports of success in the literature. The two approaches that have been explored most vigorously are transformation of B cells by Epstein–Barr virus (EBV) (2, 3) and hybridization of B cells with drug-marked mouse or human myeloma or lymphoblastoid cell lines (4–8). Difficulties in establishing stable antibody-secreting clones have been a major problem with EBV transformation, and a low frequency of hybrid clones resulting from fusion of human lymphocytes with human B-cell lines has limited progress with this approach to producing human monoclonal antibodies. Although fusion of human lymphocytes with mouse myeloma results in substantial numbers of hybrids secreting human Ig (6, 7), there is a general feeling that these interspecies hybrids are rather unstable. Nevertheless, several groups have isolated mouse–human hybrids that continued to secrete Ig for extended periods (6–8).

Edwards *et al.* (9) have recently described a hypoxanthine guanine phosphoribosyltransferase (HGPRT)-deficient human lymphoblastoid line, LICR-LON-HMy2 (LICR-2), that grows vigorously, has been shown to fuse with human lymphocytes, and produced hybrids that secrete human Ig distinguishable from the Ig of the parental line (9, 10). We report here our experience with this cell line and two other cell lines, the SKO-007 line of Olsson and Kaplan (4) and the mouse myeloma NS-1 (11).

MATERIALS AND METHODS

Cell Lines. The ARH-77-derived LICR-2 human lymphoblastoid line was kindly provided by M. O'Hare, P. Edwards, and A. M. Neville (London Branch of the Ludwig Institute for Cancer Research). The U266-derived SKO-007 human myeloma line was obtained from Becton–Dickinson (Sunnyvale, CA) and was rendered mycoplasma-free by J. Fogh (Sloan–Kettering Institute for Cancer Research). The LICR-2 line secretes κ and γ chains, and the SKO-007 line secretes λ and ε chains. The mouse myeloma line NS-1 was obtained in 1979 from U. Hämmerling (Sloan–Kettering Institute). The cells were cultured in RPMI 1640 supplemented with 7.5% fetal calf serum/1% nonessential amino acids (GIBCO)/penicillin at 100 units/ml/streptomycin at 100 μg/ml/8-azaguanine at 20 μg/ml. No growth occurred in medium containing 0.4 μM aminopterin.

Source of Lymphocytes. Sterile specimens were obtained from the Pathology Department of Memorial Hospital through the Tumor Procurement Service. Lymphocytes were derived from (*i*) regional lymph nodes (12 patients with breast cancer, 2 with lung cancer, and 1 with renal cancer); (*ii*) peripheral blood (6 patients with renal cancer and 3 normal individuals); (*iii*) spleen (4 patients with lymphoproliferative disease and 1 with renal cancer); and (*iv*) tumor specimens (4 patients with lung cancer, 4 with breast cancer, and 1 malignant pleural effusion from breast cancer).

Preparation of Lymphocytes. Tumor, lymph nodes, and spleen were freed of surrounding normal tissue under sterile conditions, minced, and passed through 500-μm cell sieves. The resultant suspension was pelleted, resuspended in RPMI 1640, layered on Ficoll-Hypaque (Pharmacia), and centrifuged at 400 $\times$ *g* for 20 min. The interface cell population was used as a source of lymphocytes for fusion. Peripheral blood lymphocytes were separated similarly on Ficoll-Hypaque gradients. Lymphocytes ($1–2 \times 10^6$ cells per ml) were incubated in RPMI 1640 medium with 7.5% fetal calf serum at 37°C for 24–48 hr prior to fusion.

Cell Fusion. Lymphocytes and the myeloma–lymphoblastoid cells were combined at a 1:1 or 2:1 ratio and washed in RPMI

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Abbreviations: LICR-2, LICR-LON-HMy2; PA, protein A; IA, immune adherence; anti-Ig, rabbit anti-human Ig; EBV, Epstein–Barr virus.

1640 and 0.2 ml of 42% (wt/vol) polyethylene glycol (M_r 4,000) [in phosphate-buffered saline containing 15% (vol/vol) dimethyl sulfoxide] was added slowly to the cell pellet with gentle mixing for 3 min at 37°C. Ten milliliters of RPMI 1640 with 7.5% fetal calf serum then was added drop by drop over a 5-min period, the cell suspension was washed and resuspended in postfusion medium (RPMI 1640/15% fetal calf serum/penicillin at 100 units/ml/streptomycin at 100 μg/ml/1% nonessential amino acids/20 μM 2-mercaptoethanol/0.1 mM hypoxanthine/16 μM thymidine). The cells were incubated overnight at 37°C, resuspended in postfusion medium containing 0.4 μM aminopterin, and plated in 96-well tissue culture plates (Costar 3596) at a density of 1–2 × 10^5 lymphocytes per well on feeder layers of BALB/c or C57BL/6 peritoneal cells (1 × 10^5 cells per well, plated 24–48 hr previously). The medium was changed once a week, and the cells were maintained in the presence of 0.4 μM aminopterin for 4–6 wk.

Immunoglobulin Detection and Quantitation. Supernatants were screened for the production of human Ig by an enzyme-linked immunoassay. Falcon 3034 plates were precoated with 10 μl of supernatant from wells containing growing clones and were incubated overnight at 4°C. The plates were washed and 10 μl of alkaline phosphatase-conjugated goat anti-human γ, μ, or α heavy chain-specific antibody (Sigma) was added to each well (1:100 dilution). After a 30-min incubation at 37°C, the plates were washed, and 10 μl of *p*-nitrophenyl disodium phosphate (1 mg/ml) in 10% diethanolamine buffer (pH 9.6) was added to each well and incubated for 30 min at 37°C. Color changes were measured by an Artek model 210 reader. The test was specific for each Ig class over a range of 500 ng/ml to 50 μg/ml. For detection of intracellular λ or κ light chains by indirect immunofluorescence (see below), goat anti-human λ or κ light chain antibodies conjugated to fluorescein isothiocyanate (Cappel Laboratories, Cochranville, PA) were used (1:40 dilution).

Serological Assays for Cellular Antigens. The protein A (PA), immune adherence (IA), and rabbit anti-human Ig (anti-Ig) erythrocyte-rosetting assays and absorption tests for the detection of cell-surface antigens and indirect immunofluorescence for the detection of intracellular antigens have been described (12–15). Rabbit anti-human Ig conjugated to fluorescein isothiocyanate (DAKO, Copenhagen) was used in the indirect immunofluorescence tests (1:40 dilution).

RESULTS

Fusion Conditions: General Comments. A number of factors in the fusion procedure were analyzed. Because of variability from fusion to fusion, firm conclusions regarding optimal conditions are difficult to reach. However, several factors were found to influence results in a generally consistent fashion. These included: (*i*) condition of myeloma–lymphoblastoid lines. The lines were maintained in logarithmic phase growth at ≥95% cell viability. Fusions with overgrown cultures resulted in a low frequency of clonal outgrowth; (*ii*) fusion ratios. Lymphocyte to myeloma–lymphoblastoid cell ratios of 1:1 or 2:1 resulted in 2–8 times greater clonal outgrowth than fusions at 5:1 or 10:1; (*iii*) time of aminopterin addition. A delay in the addition of aminopterin to the fused cells for 24 hr resulted in more vigorous growth of clones; (*iv*) fetal calf serum. Significant differences in the frequency of clonal outgrowth were found with different lots of fetal calf serum. As initially observed by Edwards *et al.* (9), some lots of serum inhibited the growth and clonability of the myeloma–lymphoblastoid cell lines and the growth of Ig-secreting clones derived from fusions. Lots of fetal calf serum therefore were prescreened for optimal growth-promoting properties by using these cell types; (*v*) other media supplements. Medium conditioned by several different cell types did not improve the frequency of clonal outgrowth. Supernatant from cultures of peripheral blood mononuclear cells stimulated 4–6 days with phytohemagglutinin and added to the postfusion medium resulted in a marked reduction in resulting clones.

Results of Fusions with NS-1, LICR-2, and SKO-007. Clones derived from NS-1 generally appeared between 2 and 4 wk after fusion, whereas clones derived from LICR-2 and SKO-007 appeared between 4 and 7 wk after fusion. All but one fusion between human lymphocytes and NS-1 resulted in growth (95%), whereas 79% of fusions with LICR-2 and 55% of fusions with SKO-007 resulted in growth (Table 1). Fusions of LICR-2 and SKO-007 with peripheral blood lymphocytes gave the poorest results, with only 60% and 40% of fusions resulting in growth, respectively. For a given number of lymphocytes, fusions with NS-1 resulted in an average of 8 times more clones than fusions with LICR-2 and >20 times more clones than fusions with SKO-007 (Table 1). There was a statistically significant difference (Student's *t* test) in the frequency of outgrowth between clones derived from NS-1 and LICR-2 ($P < 0.0005$), NS-1 and SKO-007 ($P < 0.0005$), and LICR-2 and SKO-007 ($P < 0.001$). This relationship was consistent and independent of the source of lymphocytes.

Immunoglobulin Secretion: Range and Stability. Wells with growing clones were screened for Ig secretion; 20–80% contained >500 ng of Ig per ml of supernatant. [The level of γ chain secreted by the LICR-2 line (<100 ng/ml) was generally below the sensitivity of our Ig assay. However, the possibility that the production of LICR-2-derived γ chain may be increased after fusion cannot be excluded. Human and mouse light chains and ε heavy chains were not detected in these assays.] The levels of Ig produced by the clones were similar, regardless

Table 1. Fusion frequency and percentage of Ig^+ wells: Results with three myeloma–lymphoblastoid cell lines and different sources of human lymphocytes

Fusions	Fusions with growth/ fusions done	Clones per 10^7 lymphocytes fused, no. Median	(Range)	% Ig^+ wells*	Wells screened, no.
All fusions					
NS-1	20/21	60.0	(0–250)	51	867
LICR-2	27/34	6.9	(0–74)	65	375
SKO-007	12/22	1.5	(0–26)	47	104
Lymph node lymphocytes					
NS-1	7/7	49.4	(6.6–155)	52	431
LICR-2	13/15	10.6	(0–74)	61	207
SKO-007	4/8	2.4	(0–26)	66	38
Peripheral blood lymphocytes					
NS-1	8/8	61.3	(1.5–240)	42	322
LICR-2	8/13	3.2	(0–29)	74	57
SKO-007	4/10	0.75	(0–17)	64	25
Splenocytes					
NS-1	1/1	60.0		80	60
LICR-2	4/4	4.0	(1.4–33)	70	107
SKO-007	4/4	1.6	(0.67–5.8)	20	41
Tumor-associated lymphocytes					
NS-1	4/5	46.7	(0–250)	60	54
LICR-2	2/2	11.6	(10.6–12.4)	25	4

* % of wells with growing clones having detectable levels of Ig in the supernatant (>500 ng/ml).

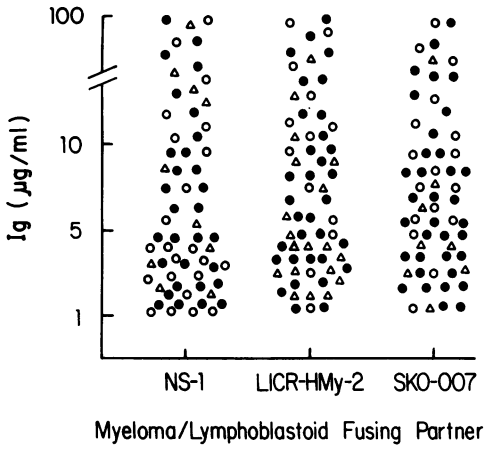

FIG. 1. Level of Ig secreted by clones derived from fusions of NS-1, LICR-2, or SKO-007 with lymph node lymphocytes (●), peripheral blood lymphocytes (○), or splenocytes (△). Ig levels in supernatant fluid of wells with growing clones were determined by an enzyme-linked immunoassay.

of the myeloma–lymphoblastoid cell line or the source of lymphocytes (Fig. 1); 70–75% of Ig-secreting clones produced between 1 and 10 μg of Ig per ml and 25–30% produced between 11 and 100 μg/ml. In 80–90% of the wells, only one class of Ig could be detected. The relative proportion of clones secreting each of the major Ig classes (IgM, IgG, IgA) was independent of the myeloma–lymphoblastoid fusion partner but appeared to be influenced by the source of lymphocytes. The results of 14 fusions are shown in Table 2. A difference was found between clones derived from peripheral blood lymphocytes and those derived from axillary lymph nodes of patients with breast cancer. A higher proportion of IgA-secreting clones resulted from fusions with axillary lymph nodes, whereas the proportion of IgM-secreting clones was generally higher in fusions with peripheral blood lymphocytes.

The stability of Ig secretion by cells derived from fusions with NS-1, LICR-2, and SKO-007 was compared over a 2- to 3-month period of subculturing, and the percentage of cultures continuing to secrete Ig was comparable (62–70%) in the case of the three fusion partners (Table 3). At 4 and 7 months after fusion, ≈50% of the cultures from NS-1 and LICR-2 fusions continued to produce Ig. Thirty-two NS-1- and 19 LICR-2-derived cultures secreting Ig at 2 months were cloned (1 cell per well) once or twice and stable Ig-secreting clones could be selected in 70–80% of the cases (observation period, ≥5 months). In our experience, the loss of LICR-2-derived Ig-secreting clones was due to cell loss rather than to instability of Ig production, because cultures derived from LICR-2 fusions were found to clone more poorly than those from NS-1 fusions.

Table 2. Ig secretion by clones derived from fusions with NS-1, LICR-2, and SKO-007: Relation to source of lymphocytes

Lymphocyte source and fusion partner	Lymphocytes fused, no.	Wells with growth, no.	% Ig⁺ wells*	% Ig⁺ wells producing μ	γ	α
Lymph node (breast cancer)						
NS-1	1.0×10^6	55	85	10	50	40
NS-1	1.0×10^7	12	67	20	10	70
NS-1	1.0×10^7	76	66	21	31	48
LICR-2	1.7×10^6	24	80	18	45	36
LICR-2	2.5×10^6	9	77	30	38	32
LICR-2	4.6×10^7	24	71	19	31	50
Peripheral blood (renal cancer)						
NS-1	2.0×10^6	48	90	8	77	15
NS-1	1.0×10^7	65	48	35	45	20
NS-1	3.7×10^6	35	49	50	50	0
NS-1	1.9×10^7	68	29	54	32	14
LICR-2	2.8×10^7	13	31	50	50	0
LICR-2	1.0×10^7	29	83	42	35	23
SKO-007	1.0×10^7	17	70	33	50	17
SKO-007	3.7×10^6	5	80	35	65	0

*% of wells with growing clones having detectable levels of Ig in the supernatant (>500 ng/ml).

Table 3. Stability of Ig secretion by clones resulting from fusions with NS-1, LICR-2, and SKO-007*

Myeloma–lymphoblastoid line	Fusions studied, no.	Cultures Ig⁺ 2–3 months after fusion†	4 months after fusion	6–7 months after fusion
NS-1	5	39/63 (62%)	30/52 (58%)	15/28 (53%)
LICR-2	7	36/51 (71%)	14/27 (52%)	14/27 (52%)
SKO-007	4	24/34 (70%)‡	—	

*Ig detectable in culture supernatant at levels of >500 ng/ml.
†Denominator indicates number of Ig⁺ cultures detected at 1–2 months after fusion and selected for further study. For results of cloning, see text.
‡Three Ig⁺ cultures from fusions with SKO-007 were subcloned. They remained stable for Ig production over a 5-month period.

Antibody Reactivity to Cell-Surface Antigens and Intracellular Antigens. The production of antibody reactive against cell-surface or intracellular antigens was tested in 422 Ig-secreting cultures by using tissue culture lines as target cells (Table 4); <1% of the cultures showed detectable antibody to cell-surface antigens, whereas 9% produced antibody to intracellular antigens.

A clone derived from a fusion of NS-1 and lymphocytes from an axillary lymph node of a patient with breast cancer (Ri37) was found to produce an IgG antibody that identifies a cell-surface antigen detected on certain cancer cell lines and on mononuclear cells from peripheral blood. The culture producing this antibody has been subcloned five times (1 cell per well) and contains both human and mouse chromosomes. It has been stable for antibody production over a 12-month period and secretes 2–5 μg of IgG per ml of culture supernatant. The antibody is de-

Table 4. Reactivity of Igs produced by cultures derived from fusions of human lymphocytes with NS-1, LICR-2, or SKO-007

Lymphocyte source	Antigen site	Fusion partner* NS-1	LICR-2	SKO-007
Lymph node	Cell surface	1/141	0/65	0/29
	Intracellular	8/141	2/65	1/29
Peripheral blood	Cell surface	1/77	1/26	0/9
	Intracellular	6/77	2/26	0/9
Spleen	Cell surface	0/30	0/41	0/4
	Intracellular	13/30	5/41	1/4

Reactivity to cell-surface antigens was tested by the PA, IA, and anti-human Ig assays. Reactivity to intracellular antigens was tested by indirect immunofluorescence. Panel of human cell lines includes (*i*) breast cancer: MCF-7, CAMA, BT-20; (*ii*) lung cancer: SK-LC-2, SK-LC-5, SK-LC-6, Law; (*iii*) melanoma: SK-MEL-41, SK-MEL-131; (*iv*) astrocytoma: U251 MG; (*v*) colon cancer: SW-1222; (*vi*) bladder cancer: 253J, TCC-SUP, Scab; (*vii*) renal cancer: SK-RC-7, KyRc; (*viii*) normal kidney: Ky, Nem.
*Number of cultures with antibody reactivity/number of Ig⁺ cultures tested.

Table 5. Absorption analysis of an IgG human monoclonal antibody (Ri37) produced by a NS-1-derived hybrid and reactive with a cell-surface antigen of normal and malignant cells

Reactive cells			
Cell line	Titer	Cell line	Titer
Lung cancer		Bladder cancer	
SK-LC-6	1:50,000	TCC-SUP	1:2,000
SK-LC-8	No titer	Peripheral blood	Tested by
SK-LC-LL	No titer	mononuclear cells,	absorp-
Melanoma		five individuals	tion only
SK-MEL-41	1:100,000	tested	
SK-MEL-151	1:1,000		

Nonreactive cells	
Lung cancer	Colon cancer
SK-LC-5, -7, -12, -13, -16	SW-1083, -1116, 1222, HT-29
Melanoma	Bladder cancer
SK-MEL-13, -19, -23, -28, -33, -37, -93-II, MeWo	J253, 639-V, Scaber
Ovarian cancer	T-cell leukemias and lymphomas
SK-OV-3	P-12, CCRF-CEM, MOLT-4, HPB-ALL, C-45
Cervical cancer	
ME-180,	B-cell lymphomas
Pancreatic cancer	SK-DHL-2, SU-DHL-10, Raji
CAPAN-2	(Burkitt), BALL-1 (B-cell
Astrocytoma	leukemia)
U251 MG, AE, AJ, AS,	EBV-transformed B cells
BD, BO, CE	BD, FG, DX, AZ, AV
Breast cancer	Erythrocytes
BT-20, AlAb, CAMA,	Fetal, newborn,
SK-BR-5, MDA-MB-157,	I^+, I^-, A, B, O, Rh^+, Rh^-, sheep
MDA-MB-231,	Normal kidney
MDA-MB-361,	KM, DZ, FO, ES, KN
ZR-75-1	
Renal cancer	
SK-RC-1, -2, -4, -6, -7, -9, -28, Caki-1	

Equal volumes of packed cells and Ri37 supernatant diluted 1:1,000–1:2,000 were mixed and incubated for 1 hr at room temperature. After removal of absorbing cells by centrifugation, residual reactivity was tested on SK-MEL-41 target cells by the anti-Ig assay.

tected by both PA and anti-Ig assays but not by IA assays. Absorption tests show that the antigen is expressed by 11 of the 87 different cell types tested (Table 5).

Thirty-eight cultures from fusions with NS-1, LICR-2, and SKO-007 have been identified that secrete antibody reactive with cytoplasmic, cytoskeletal, perinuclear, or nuclear structures. Fusion of peripheral blood lymphocytes from normal individuals as well as from tumor-bearing patients has resulted in cultures reacting with intracellular antigens. Nine of the 38 cultures have been subcloned two or more times and have remained stable for antibody production; six clones were derived from fusions with NS-1, two from LICR-2, and one from SKO-007. The reactivity of the antibodies produced by three of these clones with cultured human cells is illustrated in Fig. 2.

Characterization of Clones. Karyotypic analysis of six clones derived from NS-1 fusions with human lymphocytes and secreting human Ig showed both mouse and human chromosomes. The hybrid nature of selected LICR-2- and SKO-007-derived clones has been demonstrated by the presence of new species of light or heavy chains (or both) in the clonal population. Nine Ig-secreting LICR-2-derived clones were examined for intracytoplasmic light chain production by immunofluorescence. Three of nine clones produced a new λ light chain in addition to the κ light chain of the LICR-2 line; five produced only κ light chain and one produced only λ light chain. Three Ig-secreting SKO-007-derived clones were studied similarly. Two produced a new κ light chain in addition to the λ light chain of the SKO-007 line; one produced only the λ light chain. Analysis by $NaDodSO_4$/polyacrylamide gel electrophoresis has shown γ and κ chains in SKO-007-derived clones and μ and λ light chains in LICR-2-derived clones; these results will be described elsewhere.

DISCUSSION

Proliferating clones secreting human Ig were generated with a frequency of 1–250 clones per 10^7 lymphocytes by using the NS-1 mouse myeloma or the LICR-2 or SKO-007 human cell lines as fusion partners. Fusion with NS-1 consistently gave the highest yield of clones, 5–20 times the number obtained with LICR-2 or SKO-007. A median NS-1 fusion frequency of 60 clones per 10^7 human lymphocytes compares favorably with that of NS-1–mouse splenocyte fusions, in which the fusion frequency is 25–100 clones per 10^7 splenocytes fused. Although we expected the mouse–human hybrids to be unstable with regard to Ig production, we have not found this to be a major problem. Fifty-three percent of mouse–human hybrids followed for 6–7 months continued to secrete human Ig. Similar results were obtained with LICR-2-derived clones. The stability of Ig secretion by clones resulting from fusions with NS-1, LICR-2, or SKO-007 was found to be comparable to our experience with hybridomas derived

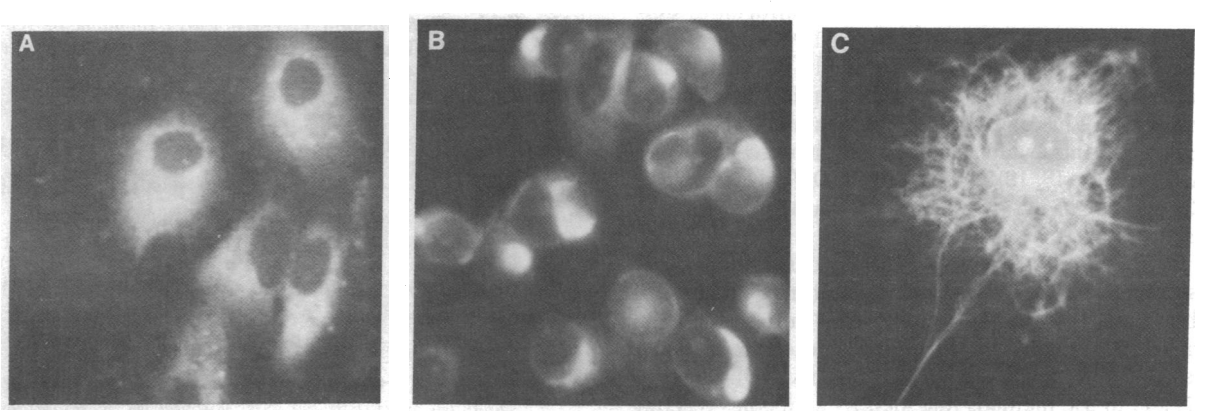

FIG. 2. Intracellular antigens of cultured human tumor cells detected by hybrids derived from fusions of human lymphocytes with NS-1. (*A*) Sm21, IgM. Lymphocyte source: lymph node, breast cancer. Cytoplasmic reactivity. (×260.) (*B*) Po71, IgM. Lymphocyte source: spleen, Hodgkin disease. Perinuclear reactivity. (×230.) (*C*) De8, IgM. Lymphocyte source: peripheral blood, renal cancer. Cytoskeletal and nucleolar reactivity. (×230.) Target cells: (*A*) Lo BR-CA; (*B*) SK-BR-5; (*C*) MDA-MB-157.

from NS-1–mouse splenocyte fusions; results from 17 recent fusions in our laboratory indicate that 53% of the mouse–mouse hybridomas remained stable for antibody secretion over a 4- to 6-month period. A key factor in maintaining Ig-secreting clones, particularly in the case of NS-1–human hybrids, is early and repeated subcloning. Non-Ig-secreting clones may have a competitive growth advantage, and without vigorous subcloning, Ig-secreting clones can be lost.

The amount of Ig produced by clones generated in this study ranged from 500 ng/ml to 100 μg/ml. Although the mechanisms regulating the levels of Ig production are unknown, it does not appear to be a feature that is conferred on the clone by the myeloma or lymphoblastoid partner, at least not in the case of the three cell lines used in this study, as Ig levels were similar for LICR-2-, SKO-007-, and NS-1-derived clones. A comparison of the source of lymphocytes also did not reveal any consistent difference in the amount of Ig produced after fusion. However, the proportion of clones secreting different Ig classes might be expected to vary with the percentage of IgM-, IgA-, or IgG-secreting B cells present in the lymphocyte source. The greater percentage of IgA-secreting clones obtained with lymphocytes from axillary lymph nodes and IgM-secreting clones with peripheral blood lymphocytes could be explained on this basis.

The hybrid character of clones derived from fusions of NS-1 with human lymphocytes has been clearly established; the presence of human and mouse chromosomes and the secretion of human Ig are unequivocal signs of a hybrid cell. The situation with clones derived from LICR-2 and SKO-007 fusions is less clear, because EBV transformation as well as hybrid formation can give rise to growing cell populations secreting human Ig. This is particularly pertinent in the case of LICR-2, which is an $EBNA^+$ lymphoblastoid line (9) and therefore a potential source of transforming EBV. Although EBV transformants are less likely to emerge in fusions with SKO-007 (which does not harbor the EBV genome), they may still arise through spontaneous EBV transformation. In theory, the distinction between EBV transformants and hybrid cells should be straightforward. EBV-transformed cell lines are reported to clone poorly (2, 3), are diploid (16), and secrete only one species of light or heavy Ig chains (or both). On the other hand, hybrid cells should clone easily, have a tetraploid DNA content, and produce (with fusion partners that secrete Ig), more than one type of light or heavy chain (or both). Experience has taught that these distinctions are not absolute. For instance, some EBV-transformed cell lines are tetraploid (17), and we have found that the majority of mouse–mouse hybridomas have a subtetraploid DNA content. In addition, human–human hybrid cells tend to be difficult to subclone and generally also have a subtetraploid DNA content. Because of problems in interpreting clonal ploidy, the most useful evidence for a human–human hybrid cell is production of distinct Ig chains. This has now been shown for a series of human–human hybrids by intracytoplasmic immunofluorescence in this study and by others (18, 19) and by $NaDodSO_4$/polyacrylamide gel electrophoretic analysis of secreted products (refs. 4 and 9; unpublished data).

With the refinement of techniques that consistently permit the construction of Ig-producing mouse–human or human–human hybridomas, there is a need to develop sufficiently broad screening methods to identify the specificity of the secreted Ig. We have chosen to initiate our screen with tissue culture lines as targets and to use methods that identify antibodies reacting with cell-surface or intracellular antigens. A tentative conclusion that can be drawn from these efforts is that human monoclonal antibodies reacting with cell-surface antigens are rare, whereas antibodies reacting with intracellular structures occur at a significantly higher frequency. Of 422 wells containing Ig-secreting clones derived from 36 individuals, <1% react with cell-surface antigens, whereas ≈9% react with intracellular antigens. Although this might indicate that there is a general prohibition against developing autoantibodies to cell-surface antigens, a larger panel of tissue culture targets would have to be tested before a definite statement can be made. It is possible that a higher frequency of surface reactivity would be seen with noncultured cells, due either to antigen loss by cultured cells or lack of a particular cell population in the tissue culture panel. A range of intracellular structures was identified by antibodies derived from lymphocytes of both normal individuals and patients with cancer, indicating that autoantibodies of this type need not be related to overt disease. Generation and detailed analysis of human monoclonal antibodies reacting with surface and intracellular antigens should give insight into the repertoire of humoral immune responses to cellular antigens and permit a definitive answer to one of the unresolved questions of human cancer immunology: Do humans develop antibodies with specificity for cancer?

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