Brief Definitive Report

HUMAN MALIGNANT AND MITOGEN-TRANSFORMED CELLS CONTAIN RETROVIRAL P15E-RELATED ANTIGEN

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Murine tumor cells (1–3) and certain murine retroviruses (4) contain factors that inhibit macrophage (MØ) accumulation at inflammatory foci. The retroviral activity is contained in the envelope protein P15E (4). P15E also inhibits lymphoproliferative responses of cats and man (5, 6). We recently showed that spontaneous and carcinogen-induced murine malignant cells synthesize a protein that is physicochemically and antigenically similar to P15E. The inhibitory activity for MØ accumulation in the tumors was removed by monoclonal anti-P15E (7). Human cancerous effusions also contain proteins that inhibit human monocyte responses to chemoattractants and the inhibitory activity was absorbed by monoclonal anti-P15E (8). We now sought to determine whether P15E-related antigens are indeed present in human malignant cells.

Materials and Methods

Cells. The cell lines studied, U937, K562, SB, MOLT-4, HL60, HL60-BII, HSB-2, and CEM, were all derived from humans with lymphoid neoplasms and obtained from the American Type Culture Collection, Rockville, MD. The U937 line was from a histiocytic lymphoma. The HL60 and the HL60 BII subclone were from an acute promyelocytic leukemia. The T lymphoblastoid HSB-2 and the B lymphoblastoid SB cell lines were from a patient with acute lymphoblastic leukemia. The T lymphoblastoid CEM and MOLT-4 lines were derived from acute lymphoblastic leukemias. The K562 line was from a chronic myelogenous leukemia effusion. All cell lines tested (HL60, HSB, CEM, MOLT-4, K562) grew as tumors in animal models. Control mononuclear cells were isolated from normal human blood with Lymphocyte Separation Media (8). Cell lines were grown in RPMI 1640 with 10% heated fetal bovine serum and antibiotics (Gibco Laboratories, Grand Island, NY).

Antibodies. Monoclonal antibodies 19F8 (IgG_{2b}) and 4F5(IgG_{2a}), which react with different epitopes on murine retroviral P15E, were contained in ascites fluid or Protein A purified from ascites or hybridoma culture supernatants (7, 9). Control antibodies were pooled normal mouse serum (NMS) or Protein A–purified IgG_{2b} or IgG_{2a} from culture supernatants of the MPC11 OUA or RPC 5.4 cell lines (American Type Culture Collection, Rockville, MD).

Indirect Immunofluorescence. Cells were fixed on ice using phosphate-buffered (pH 7.2) saline (PBS) containing 45% acetone, and 9.25% formaldehyde, washed, and incubated with antibody (final concentration = 1:40–1:100 ascites or NMS; 50.0–125.0 μg/ml

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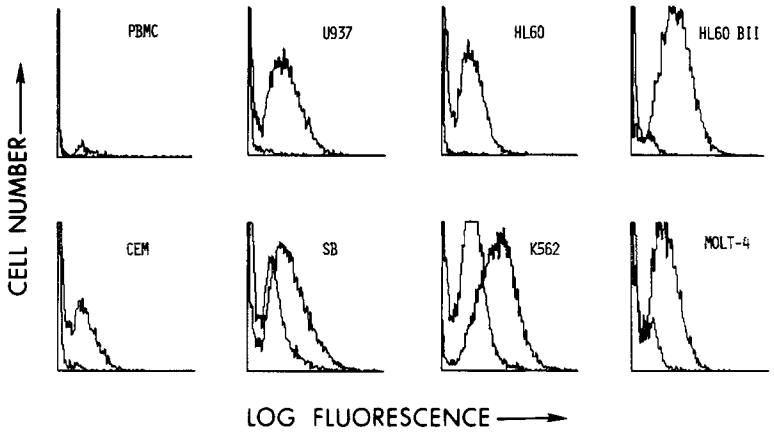

FIGURE 1. Anti-P15E reactivity of human malignant and normal mononuclear cells. Cells were fixed, incubated with 12.5 μg of anti-P15E 19F8 or IgG_{2b}, stained, and analyzed by FACS. For each cell type the less fluorescent histogram represents cells incubated with control IgG_{2b}.

TABLE I

*Fluorescence Reactivity of Monoclonal Anti-P15E Antibodies with Human Cell Lines**

Cells tested‡	n§	Antibodies used		n	Antibodies used	
		Anti-P15E, 19F8	Control‖		Anti-P15E, 4F5	Control¶
		% ± *SD*			% ± *SD*	
U937	14	74.9 ± 9.8	5.5 ± 3.8	2	70.2 ± 25.7	15.2 ± 2.3
HL60	6	56.3 ± 9.0	5.2 ± 3.5	2	64.3 ± 0.6	26.2 ± 11.8
HL60 BII	6	64.2 ± 19.1	6.8 ± 4.6	NT	—	—
CEM	5	40.4 ± 28.4	7.6 ± 8.6	NT	—	—
K562	5	79.5 ± 16.3	20.2 ± 9.4	2	57.2 ± 41.8	20.1 ± 3.2
HSB-2	3	54.6 ± 21.2	7.7 ± 7.8	2	35.0 ± 15.3	6.1 ± 4.5
SB	4	65.2 ± 14.9	18.0 ± 8.1	2	67.7 ± 16.4	14.4 ± 0.8
MOLT-4	2	76.0 ± 0.8	14.4 ± 1.6	2	29.4 ± 1.5	4.6 ± 3.5
PBMC	14	7.2 ± 4.3	1.4 ± 1.2	3	6.1 ± 10.6	1.7 ± 2.9

* Fluorescence was measured on 10,000 fixed cells as described in Materials and Methods. The photomultiplier tube setting was adjusted so that ~5% of the PBMC were fluorescent with anti-P15E. Anti-P15E(19F8) was used at a 1:40–1:100 dilution of ascites fluid or as 62.5–125.0 μg/ml of antibody isolated from ascites fluid or culture supernatants by Protein A Sepharose. Anti-P15E (4F5) was used as 50.0 μg/ml of antibody isolated from culture supernatants by Protein A Sepharose.

‡ Cells were obtained as described in Materials and Methods.

§ No. of times the cells were examined.

‖ Control antibody was either NMS used at a 1:40–1:100 dilution or 62.5–125.0 μg/ml of IgG_{2b} purified from ascites fluid or culture supernatants of the MPC11 OUA cell line.

¶ Control antibody was 50.0 μg/ml of IgG_{2a} isolated from culture supernatants of the RPC 5.4 cell line.

purified Ig) for 20 min on ice, washed, incubated on ice with FITC-conjugated goat $F(ab')_2$ anti-mouse IgG (1:100, Tago, Burlingame, CA), washed with PBS/5% goat serum, and 10,000 cells analyzed using a Coulter EPICS fluorescence-activated cell sorter (FACS). Full scale fluorescence was 1.5 log.

Mitogen Stimulation. Isolated normal mononuclear cells were resuspended to 2×10^6 lymphocytes/ml in supplemented RPMI. Cells (1 ml) were cultured with media alone or with PHA (0.5 μg/ml), Con A (5.0 μg/ml), or PWM (1:250 dilution of stock) at 37°C in a humidified, 5% CO_2 atmosphere, and prepared for immunofluorescence as above.

Reagents. Bovine serum albumin Fraction V (BSA) and dibutyrl cyclic AMP (dbcAMP) were from Sigma Chemical Co. St. Louis, MO. Phytohemagglutinin (PHA) was from

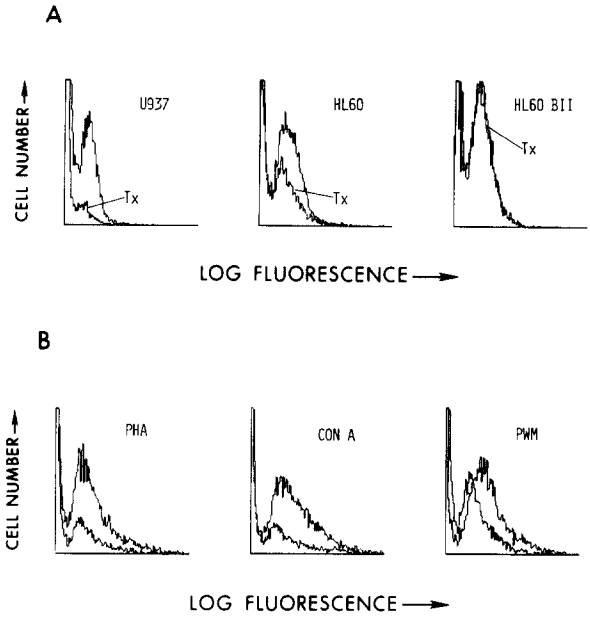

FIGURE 2. (*A*) Effect of cellular differentiation on anti-P15E reactivity. Cells were differentiated by culturing with dbcAMP, fixed, incubated with 12.5 μg of anti-P15E 19F8, stained, and analyzed by FACS. The histograms of dbcAMP treated cells are indicated by Tx. (*B*) Effect of mitogen stimulation on anti-P15E reactivity of human mononuclear cells. Cells were cultured for 48 h with the indicated mitogens, washed, fixed, incubated with 12.5 μg of anti-P15E 19F8 or IgG_{2b}, stained, and analyzed by FACS. For each mitogen the less fluorescent histogram represents cells incubated with control IgG_{2b}.

Burroughs Wellcome, Inc., Greenville, NC, concanavalin A (Con A) from Pharmacia Fine Chemicals, Piscataway, NJ, and pokeweed mitogen (PWM) from Gibco.

Results

Reactivity of Human Tumor Cell Lines with Anti-P15E. Cell lines were examined for anti-P15E reactivity by indirect immunofluorescence and FACS analysis. The photomultiplier tube setting was adjusted so that ~5% of freshly isolated mononuclear cells were reactive with anti-P15E. All cell lines reacted with the purified anti-P15E (Fig. 1 and Table I). For each tumor cell line there was a significant difference between the fluorescence of cells incubated with either of the monoclonal anti-P15E antibodies compared with those incubated with control antibodies. The difference in anti-P15E reactivity between normal mononuclear cells and malignant cells was not due to cell size (data not shown).

Effect of Cellular Differentiation on Anti-P15E Reactivity. The effects of differentiation on the ability of U937, HL60, and HL60 BII cells to bind anti-P15E was examined. Cell lines were grown in 0.5 mM dbcAMP for 48 h, conditions which induce differentiation and enhance mature leukocyte functions in the U937 and HL60 but not in the HL60 BII cell line (10–12). Treatment of U937 and HL60 with dbcAMP caused a decrease in the anti-P15E reactivity of the cells (Fig. 2*A*). In four experiments the average decreases in reactivity of the

dbcAMP-treated U937 and HL60 cells were 53.8 (37.1–85.3) and 35.8 (10.5–60.6) percent, respectively. Treatment of HL60 BII cells with dbcAMP had no effect on the anti-P15E reactivity. In five experiments the average decrease in reactivity was 3.4%. The decrease in fluorescence in differentiated cells was not due to a change in cell size, but to both decreased numbers of fluorescent cells and decreased intensity of fluorescence (data not shown).

Effect of Mitogen Stimulation on Anti-P15E Reactivity of Normal Human Blood Mononuclear Cells. Fresh mononuclear cells had little reactivity with anti-P15E. To determine the effects of blast transformation, they were cultured with PHA, Con A, or PWM for 48–72 h. The mitogens all induced the cells to react with anti-P15E (Fig. 2*B*). In four experiments the percentages of PHA- or Con A–stimulated cells reacting with anti-P15E were 58.6 (± 10.8 SD) and 37.5 (± 12.9 SD), respectively, compared with 11.4 (± 5.6 SD) and 10.0 (± 8.5 SD) with control immunoglobulin. In a single experiment the percentages of PWM-stimulated cells reacting with anti-P15E or control antibody were 46.8 and 20.3, respectively. The increase in reactivity was not due to nonspecific sticking of the antibody to the lectins (data not shown).

Discussion

All of the malignant cell lines examined reacted with two monoclonal antibodies to independent epitopes of retroviral P15E. The internal nature of this P15E-related antigen is indicated by its being detected only in permeabilized cells. The fixation process per se was unlikely to cause anti-P15E reactivity, since different fixation processes (formalin vs. glutaraldehyde-paraformaldehyde) worked equally well (data not shown). Interestingly, two other proteins associated with malignant cells, the $p60^{src}$ and p21 proteins of Rous and Harvey sarcoma virus–transformed cells, have also been reported to be internally localized (13, 14).

The loss of anti-P15E reactivity with dbcAMP differentiation of malignant cells and the increase in reactivity in mitogen-transformed mononuclear cells suggest that the synthesis of the P15E-related antigen may be controlled by a gene that is either not expressed, or is expressed at very low levels in differentiated cells, but whose expression increases during rapid cell growth. Indeed, the p53 protein has been reported in elevated amounts in a wide variety of neoplastically transformed cells and to increase in mitogen-stimulated cells (15, 16). Although we have not yet isolated and characterized the P15E-related antigen from human cells the possibility that it, like its murine counterpart, has immunosuppressive properties, would have important theoretical implications. Recent reports have indicated that human DNA contains sequences that hybridize with murine leukemia virus cDNA probes (17). Furthermore, human malignant cells contain oncogenes that are capable of inducing neoplastic transformation in vitro (18). The expression by transformed cells of additional genes that code for immunosuppressive proteins such as P15E would be likely to confer a selective advantage upon such cells for sustained growth. Thus neoplastic transformation, sufficient to allow sustained tumor growth in situ, may be a multistep process involving expression of genes regulating not only cellular transformation, but immunosuppressive properties as well.

Summary

Virus-related oncogenes have been demonstrated in human tumor cells and may play a role in neoplastic transformation. Cancerous effusions contain inhibitors of monocyte function and are absorbed by monoclonal antibodies to the immunosuppressive retroviral structural protein, P15E. We therefore examined eight human malignant cell lines for P15E-related antigens, by indirect immunofluorescence. Up to 87% of fixed malignant cells were reactive with two different monoclonal anti-P15E antibodies, while under identical conditions ~7% of freshly isolated human mononuclear cells were positive. Differentiation of two tumor cell lines with dibutyrl cyclic AMP resulted in decreased anti-P15E reactivity. Blast transformation of human mononuclear cells with mitogens induced reactivity with anti-P15E. Thus human malignant and blast-transformed cells contain antigens related to P15E. Expression of this viral-related gene may occur during rapid cell division and be abnormally regulated in cancer cells, thus rendering them more resistant to immune destruction.

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