coat in all the virion and capsid crystals implies that the structure or structures of the chromatin core can be determined by Fourier methods. Phases for x-ray reflections can be calculated to a resolution of ~30 Å (*14*) from the particle shape determined by electron microscopy (*11*), and these phases can be refined by averaging about noncrystallographic symmetry axes (*21*) to produce three-dimensional maps of the electron density within the particle. The detail that can be resolved in the chromatin core will depend on the order in the condensed nucleosome cluster and its relation to the icosahedral symmetry of the capsid.

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9. Resolution is defined by the spacing $d = \lambda/2\sin\theta$, where $\theta$ is the largest diffraction angle of the observed reflections and $\lambda$ is the x-ray wavelength. Short-range disorder in the crystals can be idealized as random displacements of the particle centers from the points of the perfect lattice. The resolution at which the Bragg reflections fade out is proportional to the mean amplitude of these displacements. In the best-ordered crystal examined, the mean displacement of the particles is about 1 percent of the nearest-neighbor separation; at the other extreme, the short-range disorder in osmium-fixed crystals, as measured by both x-ray diffraction and electron microscopy, is about ten times greater.
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## Hybrid Myelomas Producing Antibodies Against a Human Neuroblastoma Antigen Present on Fetal Brain

Abstract. *Spleen cells from mice immunized with a cultured human neuroblastoma were hybridized with the mouse plasmacytoma P3X63Ag8. Hybrid myelomas were screened for production of antibodies that reacted with human neuroblastomas but not with cells from other tissues. One of these hybridoma antibodies reacted with an antigen present on the six human neuroblastomas tested, one of two retinoblastomas, a glioblastoma, and fetal brain, but did not react with other tumors or tissues including adult human brain.*

Production of antiserums against specific antigens on human tumors or tissues has been complicated by the concomitant production of antibodies that react with all human cells. Obtaining antiserum specific for the immunizing cell has required removal of the species-specific antibodies by absorption. Köhler and Milstein (*1, 2*) have introduced methods for producing hybrid myelomas (hybridomas) that synthesize monoclonal antibodies against single antigens, thus circumventing the problem of unwanted antibodies in xenogeneic immunizations (*3-6*). We have developed a hybrid cell line that produces antibodies which react with an antigen present on human neuroblastoma, a retinoblastoma, and fetal brain. A small amount of this antigen is detectable on a glioblastoma, but it is not detectable in significant amounts on other human cell types including adult human brain.

The process of coating human tumor cells with antiserum against another human cell type has been used by other investigators (*7*) to reduce the antibody response against human species antigens and thus increase the specificity against tumor antigens. Antibody-coated cells were employed in our immunization protocol so that fewer hybridoma clones would have to be screened to find those making antibody specific for the human neuroblastomas. The human neuroblastoma line IMR6 (*8*) was incubated with a hyperimmune mouse serum directed against the human lymphoblastoid line 8866. The antibody-coated neuroblastoma cells were washed by centrifugation and injected intraperitoneally into mice. A second injection of coated cells was given intravenously 1 week later; 3 days after the second injection, the spleen cells were removed, made into a single cell suspension, and fused using polyethylene glycol 1000 with the 8-azaguanine-resistant plasmacytoma line P3-X63Ag8. Hybrids grew in HY-HAT medium (*3*) in all the wells of six 96-well plates (Linbro).

Supernatants from 200 wells were tested by microcytotoxicity (*9*) for antibodies against IMR6. Eighty-eight (44 percent) were cytotoxic to IMR6, and 59 (67 percent) of these were not cytotoxic to 8866. The hybrids producing cytotoxic antibody specific for IMR6 were passaged into tissue culture plates with 16-mm wells, and the supernatants were screened for binding to IMR6 and 8866. Antibodies bound to these target cells were detected by $^{125}$I-labeled rabbit antibody to the antigen-binding portion of the immunoglobulin (anti-Fab) (*10, 11*). The two hybrids, PI153 and PI125, which showed the highest binding activity to IMR6 with no binding to 8866 were cloned in agarose (*3*), and ten clones of each cell type were analyzed. Supernatants from these 20 clones were assayed for activity against IMR6 and all were positive. The clone from each hybrid line with the highest binding activity was then grown in culture for supernatant collection and passaged as an ascites tumor in pristane-primed mice (*3*). The supernatants from cultures that grew to more than $10^6$ cells per milliliter showed binding activity at dilutions of 1/320 or 1/640. All the ascites fluids collected from mice implanted with the cloned hybrids showed binding activity. Ascites PI153/3 exhibited binding activity to a dilution of 1/20,000. The class of antibody produced by the hybridomas


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was defined by binding the antibody to IMR6 and then detecting the bound antibody by $^{125}$I-labeled rabbit antibodies against specific classes of mouse immunoglobulins. It was determined that PI153/3 produced immunoglobulin M ($\mu$ heavy chains) and PI125/10 produced immunoglobulin G ($\gamma_2$ heavy chains).

The binding of PI153/3 and PI125/10 culture supernatants to several human cell types was detected with $^{125}$I-labeled anti-Fab (Fig. 1). Supernatants of PI153/3 reacted with all six human neuroblastomas tested, one of two retinoblastomas, a glioblastoma and fetal brain (*12*); none of the other established cell lines tested including IMR90, a human fetal fibroblast (*13*), were reactive. There was no correlation between the histocompatibility antigens (HLA-A,B) of the cells and antibody binding activity (*14*).

The absorption of the PI153/3 antibody by IMR6, 8866, and packed cell equivalents of adult and fetal human brain was measured (Fig. 2). The antigen is present in large quantities on fetal brain, even when compared to the immunizing neuroblastoma, IMR6. If reduction in antibody activity after absorption with adult brain was due to the presence of a small amount of the antigen rather than to the ratio of absorbing material to volume of antibody solution (*1, 2*), the relative amounts of antigen on adult brain, neuroblastoma, and fetal brain (calculated from the amounts of tissue necessary to absorb the same amount of antibody) would be 1, 26, and 190, respectively.

The antibody produced by PI125/10 reacted with all the cell types that bound the PI153/3 antibody except for fetal brain. It also reacted with HT1080, a fibrosarcoma; SKHep-1, a hepatoma; IMR90, a fetal fibroblast; and SCBM, a lymphoblastoid line. It also showed a low level of binding to MOLT 4, a T cell lymphoblastoid line. This pattern of reactivity could result from either activity against a polymorphic antigen or against an antigenic determinant that is present on more than one type of cell surface antigen. The molecular nature of the antigens detected by these antibodies and the possibility that PI125/10 is directed against a polymorphic antigen is being examined. The three cell types with which the antibodies from PI153/3 reacted (neuroblastoma, retinoblastoma, and glioblastoma) are embryologically derived from neuroectoderm (*15*). The fact that the antigen is present on fetal brain in high concentration but absent or at much lower concentration on adult brain suggests that this antigen is specific for an early stage in the development of the nervous system.

These results indicate that the use of hybridomas for the production of antibodies against tumor specific antigens and differentiation antigens is an important new approach toward identifying and isolating these cell surface components. The production of a series of monoclonal antibodies against cells from different nervous system tumors as well as against normal nervous tissue should prove useful in defining the lineage and fate of cells in the nervous system. Such antibodies could be used to identify distinct subpopulations of cells, to determine the locations of these cells, and to isolate the membrane components that serve as markers of specific stages of nervous system development. In addition, it has been suggested that drugs can be covalently attached to tumor-specific antibodies and then delivered specifically to tumors (*16*). The availability of large amounts of monoclonal antibodies that react with tumors but not with normal adult cells may make this a practical form of therapy.

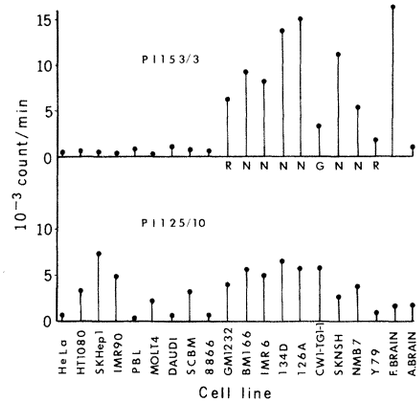


Fig. 1. Binding of PI153/3 and PI125/10 antibodies to human cells. Between $0.5 \times 10^6$ and $1 \times 10^6$ of each cell type, or an equivalent packed cell volume of homogenized brain was incubated for 1 hour at 4°C with 25 $\mu$l of supernatant or diluted ascites fluid. The cells were washed, and $^{125}$I-labeled anti-Fab was added (100,000 count/min). After incubation, the tubes were washed twice and counted. Samples were done in duplicate. The neuroblastomas (N) tested were 134D, IMR6, 126A, BM166, SKNSH, NMB7; the retinoblastomas (R) were GM1232, Y79; and the glioblastoma (G) was CW1-TG1-1 (A. McMorris, Wistar Institute). Other cell lines were fibrosarcoma HT1080 (C. Croce, Wistar Institute); hepatoma SKHep-1 (J. Fogh, Sloan Kettering); fibroblast IMR90 (Institute for Medical Research, Camden); B lymphoblastoid lines Daudi, SCBM, 8866; T cell line MOLT4; Peripheral blood lymphocytes PBL; and HeLa cells D98-AH/2. The same background binding was found with whole adult human brain or with separate preparations of grey or white matter from cerebellum or cerebrum.

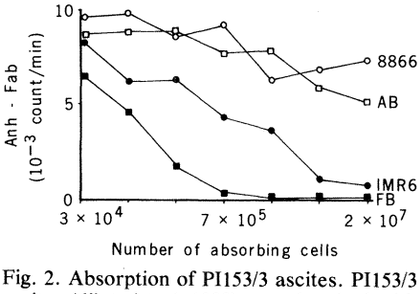


Fig. 2. Absorption of PI153/3 ascites. PI153/3 ascites (diluted to 1:1000) was absorbed with 8866, a B lymphoblastoid line; IMR6, a neuroblastoma; and packed cell equivalents of homogenized human adult brain and fetal brain; and the binding of the absorbed antibody solution was tested against IMR6. The indicated number of cells at a 1:3 dilution, suspended in a final volume of 50 $\mu$l, was incubated with 50 $\mu$l of ascites fluid at a dilution of 1:1000 for 1 hour at 4°C. The cells were removed by centrifugation and the antibody remaining in the supernatant was detected by binding to IMR6 using the binding assay described in Fig. 1. The ratio of packed cell volume to liquid at the highest concentration of cells was 1:2.


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13. IMR90 is a fetal fibroblast obtained from the Institute for Medical Research, Camden, N.J.
14. PI153/3 and PI125/10 were tested against a panel of HLA-A and -B typed peripheral blood lymphocytes. There was no reactivity with any of these cells. Most of the cell lines used were typed by microcytotoxicity for HLA-A and -B with a panel of 60 HLA serums, and there was no correlation between HLA-A and -B type and reactivity of the hybridoma antibodies.
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