the viral lytic cycle—were not observed. It seems therefore that fresh human epithelial cells may, like human B lymphocytes, have control mechanisms that suppress the EBV lytic cycle. This suppression, concomitant with the expression of EBNA and induction of cellular DNA synthesis, is a prerequisite for the transformation of human B cells by EBV (*1, 3*). EBNA is observed in 95 percent of Burkitt's lymphona cases and in virtually all cases of undifferentiated nasopharyngeal carcinoma (*1, 3*). Thus the detection of EBNA in EBV-infected normal human epithelial cells strengthens the case for a role of EBV in the etiology of nasopharyngeal carcinoma.

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Stable Antibody-Producing Murine Hybridomas

Abstract. *A method is described for obtaining antibody-producing hybridomas that are preferentially retained in cultures of fused mouse spleen and myeloma cells. Hybridomas are produced by fusing mouse myeloma cells that are deficient in adenosine phosphoribosyltransferase (APRT) with mouse spleen cells containing Robertsonian 8.12 translocation chromosomes. The cell fusion mixtures are exposed to a culture medium that can be utilized only by APRT-positive cells, which results in the elimination of both unfused APRT-deficient myeloma cells and non-antibody–producing APRT-deficient hybridomas that arise by segregation of the 8.12 translocation chromosomes containing the APRT genes and the active heavy chain immunoglobulin gene.*

Cell fusion mixtures containing hybrids of mouse spleen and myeloma cells (hybridomas) are cultured in HAT medium (hypoxanthine, aminopterin, and thymidine) for 10 to 14 days to eliminate unfused hypoxanthine phosphoribosyltransferase (HPRT)–deficient myeloma cells (*1–9*). This selection system, however, can eliminate hybridomas that segregate the spleen X chromosome encoding the active HPRT locus (*9, 10*). For $HPRT^+$ antibody-producing hybridomas to survive in HAT medium they must retain the spleen X chromosome that encodes the active HPRT locus and the specific spleen autosomes that contain the active structural immunoglobulin (Ig) chain loci (chromosome 6, light chain kappa; or chromosome 16, light chain lambda; and chromosome 12, heavy chain) (*11, 12*).

One of us (R.T.T.) conceived a hybridoma selection strategy that permits ascertainment of the contribution of X chromosome segregation toward loss of hybridomas (Fig. 1) (*13*). In the Robertsonian (8.12) 5Bnr mouse [Rb(8.12) mouse] (*14*), the active heavy chain Ig locus on chromosome 12 (only one allele of the diploid complement is expressed) and a selectable enzyme marker locus (adenosine phosphoribosyltransferase, APRT) on chromosome 8 (*15, 16*) are genetically linked. Thus, the exposure of cell fusion mixtures to a medium requiring APRT activity ($APRT^+$ selection) eliminates both unfused $APRT^-$ myeloma cells and $APRT^-$ hybridomas. The latter are incapable of heavy chain Ig synthesis because of segregation of the 8.12 translocation chromosomes. For antibody-producing hybridomas to survive this selection procedure they must retain only the spleen chromosomes that contain the structural Ig chain loci. In this report we describe the production of hybridomas secreting antibodies for human pepsinogen I, and present evidence that during selection in HAT medium X chromosome segrega-

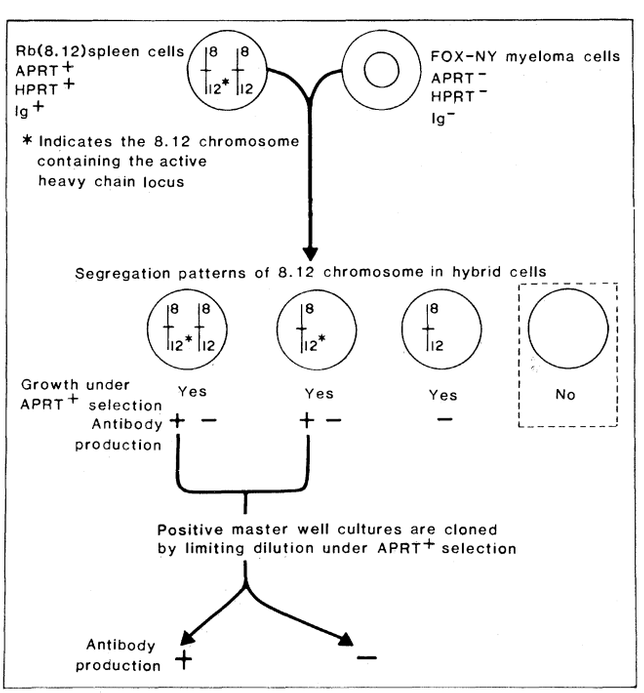

Fig. 1. Hybridization of Rb(8.12) spleen cells with FOX-NY myeloma cells and isolation of antibody-producing hybridomas under $APRT^+$ selection. The $APRT^+$ selection media (see text) contained azaserine to block de novo purine synthesis or aminopterin to block de novo purine and pyrimidine synthesis [for a review, see (*16*)]. Hybridomas that retain one or both 8.12 translocation chromosomes also produce APRT enzyme activity, which is necessary for survival under $APRT^+$ selection. Hybridomas that lose both 8.12 chromosomes as well as unfused $APRT^-$ myeloma cells are unable to utilize exogenous adenine to synthesize purines; exposure to $APRT^+$ selection medium eliminates them within 36 hours.

tion causes the loss of large numbers of hybridomas.

The myeloma cell line designated FOX-NY was isolated as a spontaneous mutant of NS-1 (*8*). It is resistant to the effects of toxic analogs of adenine (2,6-diaminopurine, $10^{-4}M$) and hypoxanthine (8-azaquanine, $10^{-4}M$), is doubly enzyme deficient ($APRT^-$ and $HPRT^-$) (*17–19*), and is eliminated during $APRT^+$ or $HPRT^+$ selection (*15, 16*). Cytogenetic analysis of trypsin-Giemsa–banded metaphase preparations revealed a heterogeneous cell population that contained an average of 61 acrocentric chromosomes (range, 45 to 65) and one submetacentric chromosome (range, 0 to 5). To date no $APRT^+$ or $HPRT^+$ revertants have been observed from this cell line.

Using the procedure described by Oi and Herzenberg (*5*), we produced hybridomas by fusing 6.2×10^7 spleen cells from a female Rb(8.12) mouse previously immunized with a purified preparation of human pepsinogen I (*20, 21*) with 1.4×10^7 FOX-NY cells. The cell fusion mixture was dispensed into 240 individual microcultures at a density of 3.2×10^5 cells per well (Costar 96-well culture plates; 0.12 ml, 2.7×10^6 cells per milliliter). After incubation overnight in nonselective medium [Dulbecco's minimum essential medium (DMEM) containing 15 percent fetal bovine serum, Flow Laboratories], microcultures were fed one of two selection media that could be utilized only by $APRT^+$ cells ($APRT^+$ selection) (*15, 16*): 160 received AAT selection medium ($7.5 \times 10^{-5}M$ adenine, $8 \times 10^{-7}M$ aminopterin, and $1.6 \times 10^{-5}M$ thymidine) and 80 received AsA selection medium ($10^{-6}M$ azaserine and $7.5 \times 10^{-5}M$ adenine). The microcultures were maintained at 37°C with 7 percent CO_2 and were refed at 2- to 3-day intervals. At 14 days, 124 of 160 AAT microcultures and 52 of 80 AsA microcultures contained antibody to pepsinogen I, as determined by solid-phase radioimmunoassay (*22*).

Positive cultures were expanded and either cloned directly by limiting dilution on feeder layers of Balb/c thymus cells or stored under liquid nitrogen for later study. Clones were screened for antibody production by polyacrylamide gel electrophoresis (Fig. 2). Antibody-producing clones have been obtained from 89 of 104 original microcultures examined thus far and 12 of these microcultures contain more than one type of hybridoma. We estimate, therefore, that the original 176 microcultures contained more than 200 antibody-producing hybridomas.

Each of 16 antibody-producing clones examined contained cellular APRT activity (*17*) and one or both copies of the 8.12 translocation chromosome (mean, 1.4 copies per cell). When these hybridomas were exposed to $APRT^-$ selection medium (DMEM containing 15 percent fetal bovine serum and $10^{-4}M$ diaminopurine) after several passages in nonselective medium, only nonantibody–producing $APRT^-$ hybridomas were obtained. These hybridomas were eliminated upon subsequent exposure to $APRT^+$ selection medium. Clones maintained in $APRT^+$ selection medium or normal medium for similar periods of time (3 to 6 weeks), however, continued to produce antibody. These observations are consistent with the expectation that $APRT^+$ selection promotes the growth of those hybridomas retaining the heavy chain Ig locus (Fig. 1).

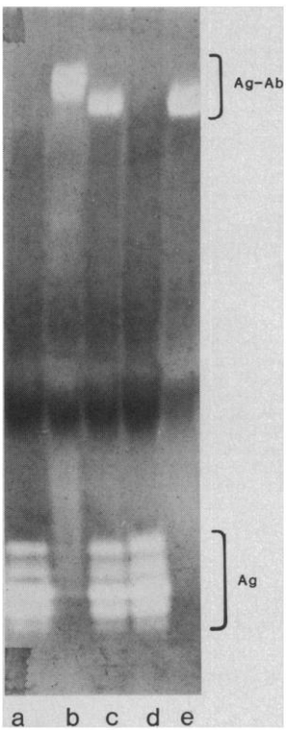

Fig. 2. Polyacrylamide gel immunoabsorption analysis. Supernatants from hybridoma clones obtained from the same antibody-positive microculture were incubated with human urinary pepsinogen I isozymes (1 μg/ml) in 20 percent sucrose overnight at 4°C, subjected to polyacrylamide electrophoresis and stained for proteolytic activity (*19, 20*). Culture medium (a) and an antibody negative clone (d) exhibit no retardation of antigen (*Ag*). Antibody-positive cultures exhibit partial retardation (b and c) or complete retardation (e) of the antigen. The different rates of anodal migration of the antigen-antibody complex (*Ag–Ab*) in (b) versus (c) and (e) suggest that at least two different hybridomas were present in the original microculture.

We examined 80 antibody-producing clones for HPRT activity to estimate the degree of spleen cell X chromosome loss. Fifty-one (64 percent) examined were $HPRT^-$ (*17*), suggesting that most of the hybridomas would have been eliminated under $HPRT^+$ selection. The relative survival of hybridomas during initial culture under $APRT^+$ or $HPRT^+$ selection conditions was examined in a second experiment. We fused 9.9×10^7 spleen cells from a female Rb(8.12) mouse with 2.5×10^7 FOX-NY myeloma cells and dispersed the cell fusion mixture in nonselective medium (3.2×10^5 cells per well). After they were incubated overnight, an equal number of microcultures were exposed to either AAT or HAT ($1 \times 10^{-4}M$ hypoxanthine, $8 \times 10^{-7}M$ aminopterin, and $1.6 \times 10^{-5}M$ thymidine) medium. Four days later the number of hybridoma colonies per microculture well differed markedly between the two selection systems (Fig. 3). There were a total of 1112 hybridoma colonies per 100 microcultures exposed to AAT and 697 colonies per 100 microcultures exposed to HAT medium. That 37 percent fewer hybridomas survived HAT selection compared to AAT selection after only 4 days of

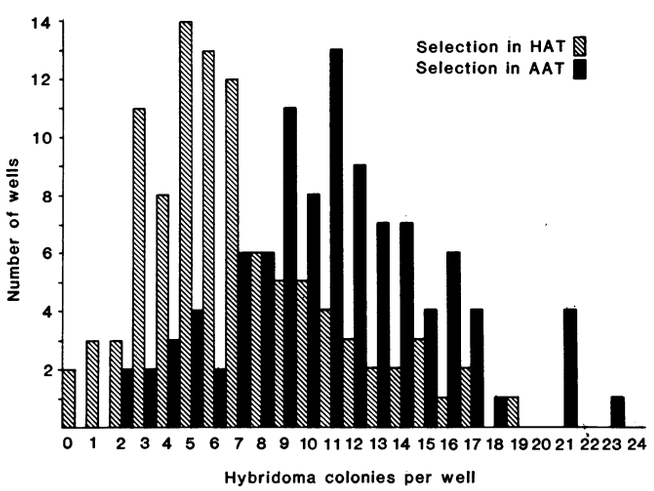

Fig. 3. Hybridoma colonies per microculture after 4 days of exposure to HAT or AAT medium. From a total of 384 microcultures hybridoma colonies were counted in 200 cultures by using an inverted phase-contrast microscope. Only colonies containing more than 16 cells were recorded. No differences in growth rates were observed when cultures of $APRT^+/HPRT^+$ hybridomas were exposed to HAT or AAT medium.

culture provides further evidence that hybridomas are eliminated from cell fusion cultures exposed to HAT medium as a result of X chromosome segregation.

The loss of spleen cell chromosomes is an important cause of unstable antibody production by hybridomas (*1–3, 5–8*). Previous studies have demonstrated that the heavy chain synthesis is preferentially lost before light chain synthesis and that these losses correlate with segregation of the spleen cell chromosomes encoding the genes for the respective Ig chains (*3, 11, 12*). The hybridoma selection method described here, however, promotes the growth of those hybridomas which retain the spleen cell–donated heavy chain Ig gene and eliminates the loss of large numbers of hybridomas caused by X chromosome segregation during culture in HAT medium. This phenomenon may have contributed to the limited success in producing human lymphocyte–human myeloma hybridomas (*23*).

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21. A female Rb(8.12) mouse was immunized with a purified preparation of human pepsinogen I as follows: one intraperitoneal injection of 80 μg in complete Freunds adjuvant and another of 50 μg in incomplete Freunds adjuvant 3 weeks later, then an intravenous (via tail vein) injection of 20 μg in saline was given 4 weeks later.
22. Microwell cultures were assayed by incubating 100 μl of ^{125}I-labeled human pepsinogen I (2×10^4 count/min) with 100 μl of the respective culture supernatant in 96-well polyvinyl chloride plates coated with human pepsinogen I for 2 to 3 days at 4°C. Positive cultures immobilized between 3 and 51 percent of ^{125}I-labeled antigen.
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An Excitatory Amino Acid Antagonist Blocks Cone Input to Sign-Conserving Second-Order Retinal Neurons

Abstract. *cis-2,3-Piperidinedicarboxylic acid (PDA), an excitatory amino acid antagonist, reversibly blocked cone input to OFF bipolars and horizontal cells, whereas ON bipolars were relatively unaffected. Kainic acid effects were also blocked, indicating a postsynaptic mechanism of action. The use of PDA helps to characterize one of two classes of excitatory amino acid synaptic receptors that mediate cone influence in the outer retina.*

The photoreceptors of the vertebrate retina form chemical synapses on three classes of second-order neurons: horizontal cells, ON bipolars, and OFF bipolars (*1*). A single photoreceptor may synapse on two or possibly all three types (*2*). For this reason, it is widely accepted that a single transmitter activates all three second-order neurons. Aspartate and glutamate have been the prime photoreceptor transmitter candidates since they mimic the endogenous transmitter when applied to all three cell types in several vertebrate species (*3*). In the mud puppy retina, one of these second-order neurons, the ON bipolar, contains a distinct synaptic receptor that binds to 2-amino-4-phosphonobutyrate, a glutamate analog (*4*). This selectivity has been confirmed in several vertebrate species (*5, 6*). The one-transmitter hypothesis requires that the other two second-order neurons also have excitatory amino acid synaptic receptors, although differing from that of the ON bipolar. We now report on the effects of an excitatory amino acid antagonist, (±)-*cis*-2,3-piperidinedicarboxylic acid (PDA), which acts on the horizontal cell and the OFF bipolar and thus helps to characterize this other receptor type.

Experiments were performed in the light-adapted, superfused retina-eyecup preparation of the mud puppy *Necturus maculosus*. Intracellular recordings were obtained from retinal neurons while an amphibian Ringer superfusate containing a control solution was interchanged with one or more solutions containing pharmacological agents. Rapid exchange in this system permits detectable drug effects within 10 to 25 seconds. The light stimulus consisted of small spot (200 μm), annulus (inner diameter, 400 μm; outer diameter > retinal diameter), or full-field illumination. Changes in input resistance were frequently monitored with a ± 0.1-nA current pulse applied through the electrode and a bridge-balancing device [WP707; see (*7*) for details of methodology].

When PDA, in concentrations up to 10 m*M*, was applied to the retina (Fig. 1a), there was no apparent effect on cone photoreceptors (*8*). In addition, PDA did not block synaptic transmitter release from photoreceptors because ON bipolars (Fig. 1b) functioned normally in the presence of these high levels of PDA.

In contrast to the photoreceptors and ON bipolars, the horizontal cells and OFF bipolars were very sensitive to PDA. Horizontal cells and OFF bipolars are hyperpolarized in the light as a result of a decrease in transmitter release from photoreceptors (*9*). Horizontal cells were also hyperpolarized by PDA (Fig. 1c), and there was a corresponding decrease