(NADH), hydroxypyruvate (2.5×10^{-4} to $2 \times 10^{-3}M$) markedly stimulated the conversion of glyoxylate to oxalate and decreased the reduction of glyoxylate to glycolate (Fig. 3). This effect was observed equally with preparations of lactate dehydrogenase from beef heart and rabbit muscle. Pyruvate in comparable concentrations also stimulated oxalate synthesis but *p*-hydroxyphenylpyruvate had no effect. At concentrations of hydroxypyruvate greater than $5 \times 10^{-3}M$ a decrease in the stimulation of oxalate synthesis was observed.

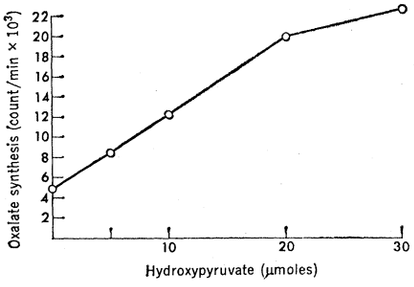

Fig. 2. Effect of various concentrations of hydroxypyruvate on synthesis of oxalate from glyoxylate in human erythrocyte-hemolyzate system. Reaction mixture contained 1.5 μmole of sodium [1-^{14}C]glyoxylate (254,000 count/min per micromole), 100 μmole of phosphate buffer (*p*H 7.4), and erythrocyte hemolyzate containing 20 mg of hemoglobin in a final volume of 1.0 ml. Mixture was incubated for 30 minutes at 37°C; reaction was stopped with addition of 0.1 ml of 1*M* citric acid, and decarboxylation of formed [^{14}C]oxalate was carried out by addition of purified fungal oxalate decarboxylase (*3*) with trapping of $^{14}CO_2$ in Hyamine for liquid scintillation counting.

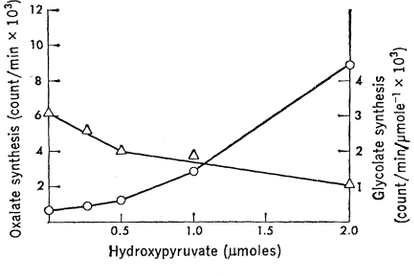

Fig. 3. Effect of various concentrations of hydroxypyruvate on synthesis of oxalate and glycolate from glyoxylate catalyzed by lactic dehydrogenase. Reaction mixture contained 0.75 μmole of sodium [1-^{14}C] glyoxylate (254,000 count/min per micromole), 100 μmole of phosphate buffer (*p*H 7.4), 0.5 μmole of NADH, 5 μg of beef heart lactate dehydrogenase type III (Sigma) in a final volume of 1.0 ml. The mixture was incubated for 30 minutes at 37°C. Oxalate was determined as described in Fig. 2. Glycolate synthesis was determined by addition of 20 μmole of carrier glycolate at the end of the incubation period followed by ion-exchange chromatography on Dowex-1-acetate, paper chromatography, and determination of radioactive glycolate with 2,7-dihydroxynaphthalene and liquid scintillation counting (*4*). ○, Oxalate synthesis; △, glycolate synthesis.

These data indicate that hydroxypyruvate is capable of stimulating the oxidation of glyoxylate to oxalate in a coupled reaction catalyzed by lactate dehydrogenase (LDH) in the presence of NADH, as reported for pyruvate (*6*). These observations offer an explanation for the increased synthesis and excretion of oxalate in L-glyceric aciduria, in which the defect in D-glyceric dehydrogenase presumably leads to the intracellular accumulation of hydroxypyruvate (Fig. 1). This effect of hydroxypyruvate apparently results from the increased oxidation of the LDH-NADH complex secondary to the reduction of hydroxypyruvate to L-glycerate. This shift in the ratio NAD:NADH may also explain the concomitant reduction in glycolate synthesis from [^{14}C]glyoxylate observed in these patients (*1*). The decrease in the stimulation of oxalate synthesis at higher concentrations of hydroxypyruvate may be related to competition of hydroxypyuvate with glyoxylate for catalytic binding sites on the enzyme.

This explanation for the increased oxalate synthesis found in L-glyceric aciduria represents a novel mechanism for the phenotypic expression of a human genetic disease. The recent description of a patient with chronic lactic acidosis associated with an altered reduction-oxidation state of several NAD:NADH-coupled reactions suggests a somewhat analogous but more generalized metabolic derangement, although no specific enzymatic defect was found (*7*). It can also be anticipated that the accumulation of other keto acids which are substrates for lactate dehydrogenase might secondarily result in hyperoxaluria and hypoglycolic aciduria. Such an additional patient has been found and this explanation is being pursued (*8*). Finally, this further emphasis of the important role of lactate dehydrogenase in oxalate synthesis in man suggests a possible site for the development of metabolic inhibitors. An effective inhibitor of oxalate synthesis would allow a rational approach to the treatment of primary hyperoxaluria, a serious and often fatal group of diseases.

HIBBARD E. WILLIAMS
LLOYD H. SMITH, JR.
Department of Medicine, University of California School of Medicine, San Francisco 94122

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Selective Stimulation of Allelic Expression: Effect of Antibodies to Allotypic Markers on Lymphoid Cells

Abstract. *Peripheral blood leukocytes from rabbits which were heterozygous* (b^5/b^9) *for markers on their immunoglobulin light chains were maintained in vitro for up to 24 hours in the presence or absence of antibody to b9. After culture they were transferred into lethally irradiated* b^4/b^4 *hosts. Recipients of cells exposed to antibodies to allotype markers showed a striking increase in concentration of circulating b9 molecules and number of b9 plasma cells in their spleens compared to control animals receiving untreated cells from the same donor. There was no appreciable difference between the two groups of recipients with respect to their content of b5 molecules and immunocytes.*

Allogeneic transfer of lymphoid cells into lethally irradiated recipient rabbits has been used to assess the potential of the donor cells to proliferate and differentiate into immunocytes (*1*). The donor or recipient origin of the antibody-synthesizing and secreting cells has been established by identification of the genetically determined allotypic markers on the κ light chains of their product immunoglobulins (*2*). Our assay for precursors of immunocytes in the

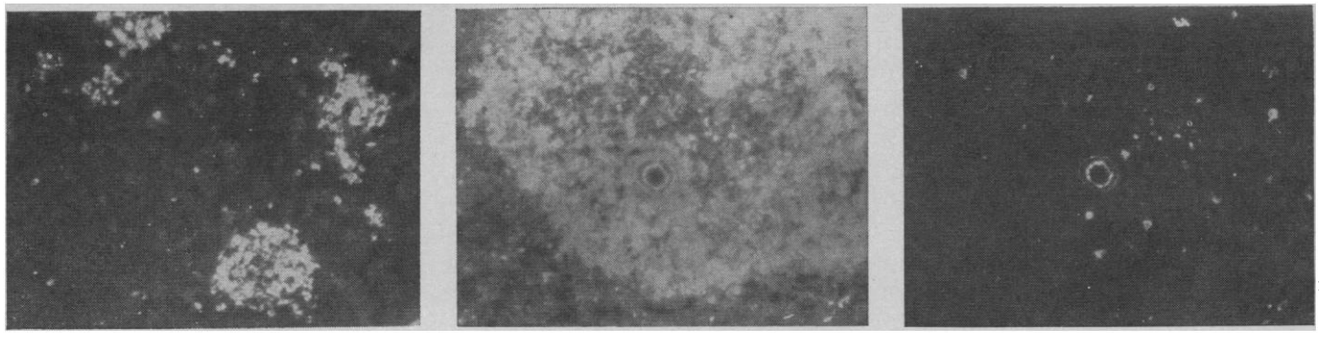

Fig. 1. Clusters of immunocytes in the repopulated spleens of irradiated b^4/b^4 rabbits given cultured cells from b^5/b^9 donors 6 days previously (× 125). All sections were stained with rhodamine (red) labeled antibody to b5 and fluorescein (green) labeled antibody to b9. (Left) Section from a recipient of untreated cells. Exciting light passed through green interference filter ($T_{max} = 546$ nm; Schott), and fluorescence was visualized through red (RG1) Schott filter. Only b5 cells can be seen under these conditions but few if any b9 cells were present in the clusters shown. (Middle) A very large green-fluorescing b9 cluster around a blood vessel in the spleen of the recipient of cells exposed to antibody to b9. The exciting light passed through a blue (BG12, Corning) filter, and the fluorescence was observed through a yellow filter (K490, Schott). (Right) Same field as shown immediately above except red, rhodamine-stained b5 cells only are apparent. The illumination is the same as used for the top field. This large cluster contains an overwhelming majority of b9 marked cells.

rabbit, like assays in which mice were used (*3*), is based on the detection of groups of antibody-forming cells among those cells repopulating the spleens of irradiated recipients. However, in our analysis of single cell suspensions and thin sections from the recipients' spleens by immunofluorescence methods, we are able (i) to enumerate immunocytes that make each polymorphic form of immunoglobulin and (ii) to map the distribution of these differentiated cells within the repopulated spleen, regardless of the antibody specificity of their product (*4*). Using this assay we have found that peripheral blood leukocytes maintained in vitro for up to 30 hours still include immunocyte precursors. Furthermore, if the cell donor is heterozygous at the κ chain (*b*) locus, exposure of the peripheral blood leukocytes to antibodies directed against only one of the allelic parental markers results in a striking increase in cells containing that marker and in an increase in the amount of immunoglobulins produced which bear that marker in the recipient animals. Ordinarily, immunocytes show allelic exclusion with respect to the allotypic markers, each cell of a heterozygous animal producing molecules of only one parental allotype (*5*). Thus, exposure of cultured cells to an antibody to an allotypic marker seems to prejudice the outcome of their subsequent proliferation or differentiation in favor of a higher yield of cells that make the corresponding allelic product.

Blood samples (30 to 87 ml) were drawn from the marginal ear vein of three b^5/b^9 heterozygous donors into Hanks balanced salt solution containing 4000 international units (I.U.) of heparin. The blood elements were sedimented at 4°C and washed three times with Eagle's minimal essential medium (MEM) containing penicillin (100 I.U./ml) and streptomycin (100 μg/ml). Cultures of unseparated blood elements were set up in flat, Falcon culture flasks (250 ml, 75 cm^2, disposable) to initially contain 5 to 10 × 10^7 white blood cells in 50 ml of fluid. The cells were cultured in MEM containing 1 percent (by volume) Antibiotic-Antimycotic solution (Grand Island) and 10 percent (by volume) of sterile b^4/b^4 rabbit serum or fetal calf serum (in which the complement was inactivated by heat), both previously dialyzed against MEM. Finally, concentrated rabbit immunoglobulin G (IgG) from b^4/b^4 donors, containing antibodies to b9, was added in varying amounts to some of the cultures after it was first dialyzed against MEM and sterilized. After the culture period the cells were sedimented and washed three times in MEM. Cell suspensions containing 1 to 3 × 10^6 nucleated cells per milliliter were prepared in MEM.

The desired number of cells were injected intravenously into b^4/b^4 recipients that had been exposed to 1000 roentgens x-irradiation 24 hours earlier. The host rabbits received injections of 100 mg of streptomycin and 2 × 10^6 I.U. of penicillin daily. After the period of culture in vivo, the recipient rabbits were exsanguinated. Their spleens were each cut transversally into 2- to 4-mm fragments, and alternate pieces were combined and used for the preparation of a single-cell suspension. Portions of this suspension, each containing 2 × 10^5 leukocytes, were used to deposit replicate films on microscope slides with a cytocentrifuge (*6*). The remaining spleen fragments were quick-frozen in a tube containing isopentane and immersed in a Dry Ice–acetone bath, and sectioned (2 to 4 μm) in a cryostat microtome. The cell films and tissue sections were stained with a mixture of fluorescein-labeled IgG from antiserum to b9 and tetramethylrhodamine-labeled IgG from antiserum to b5, both prepared in b^4/b^4 rabbits (*7*). The absolute concentrations of b9 and b5 molecules present in the serums of recipients at the time of killing were determined by quantitative precipitin analysis, in which IgG's from appropriate homozygous animals were used as standards.

The b9 molecules in the cell donors' serums, obtained when blood was taken for culturing, comprised 22, 25, and 23 percent of the total b5 + b9 molecules for rabbits Nos. 3, 4, and 5, respectively. Although these donors were not killed, other studies indicate that the proportion of immunocytes synthesizing each of two allelic immunoglobulins in heterozygotes closely reflects the relative serum concentrations of their products (*8*). Table 1 shows that in all seven of the surviving positive recipients that received cells cultured in the absence of antibody to allotypic marker, b5-producing immunocytes predominated in the spleen and there were more b5 molecules in the circulating blood, as compared to b9 marked cells and immunoglobulins. The percentage of donor cells bearing the b9 marker ranged from 17 to 37 and the percentage of b9 molecules relative to total donor molecules ranged from 28 to 36. Thus recipients of untreated cells resembled the donors in having a predominant

population of b5 cells and molecules relative to those with the other allelic marker. In contrast to the above, the six surviving positive recipients of peripheral blood cells that were cultured in the presence of antibody to b9 showed an exaggerated proportion of b9 cells and circulating b9 product compared with the cell donors and control recipients (Table 1). The percentage of b9 molecules in the serum ranged from 58 to 93 percent in these animals, and the proportions of b9 cells varied from 48 to 83 percent. Thus in animals receiving cells treated with antibody to b9, the proportions of b5 and b9 cells and molecules were reversed as compared with control recipients and donors. Furthermore, recipients of cells treated with antibody to b9 tended to have a higher concentration of circulating b9 molecules than control recipients killed at the same time. In contrast, most recipients of treated or untreated cells from a given donor had approximately the same concentration of b5 molecules in their serum at a given time.

Productive cells containing each marker are harder to determine quantitatively on an absolute basis than circulating molecules because the total number of cells deposited by the cytocentrifuge tends to vary. Thus the greater number of cells producing b9 molecules expected in recipients of cells treated with antibody to b9 cannot be convincingly represented by the absolute counts of immunocytes on cytocentrifuge films. However, stained sections show the differences between numbers of immunocytes of a given allotype in the spleens of the recipients of either control or treated cultures. Figure 1 depicts typical clusters of immunocytes found in the spleens of control and experimental recipients. The numbers of clusters of immunocytes containing different proportions of b9 and b5 cells on three cross sections of each spleen—taken from different regions—are tabulated in Table 1. While the spleens of recipients of untreated cells had a majority of clusters in which b5 cells predominated, the spleens from recipients of cells treated with antibody to b9 showed a striking profusion of b9 cells. The b9 cells in the spleens of this group of experimental recipients occurred in many clusters, some of great size. Most of the clusters were relatively homogeneous with respect to cells making one or the other allelic marker (Table 1) (Fig. 1). By day 6, there was no difficulty in recognizing sections as being derived from the control or experimental group

Table 1. Proportions of b5 and b9 splenic immunocytes and serum molecules found at the time of killing in b^5/b^5 recipients of cultured peripheral blood from b^5/b^9 donors.

Recipient*	Donor No.	Inoculum†		Day of killing	Serum conc. of donor Ig's‡ (μg/ml)			Cell counts on cytocentrifuge film§			No. of clusters with different percentage of b9 cells ‖					
		No. of WBC‡ ($\times 10^7$)	Anti-b9 in culture (mg)		b5	b9	b9 (%)	b5	b9	b9 (%)	Small (<75 cells)			Large (>75 cells)		
											>90	50–90	10–50	<10		
417	3	3.5	0	6	58	27	32	1106	436	28	9	6	10	38	1	6	4	8
418	3	3.5	0	6	34	18	35	291	150	34	10	8	9	26	0	4	1	11
419	3	3.5	0	6	23	12	34	258	65	20	0	1	0	20	0	0	0	8
420	3	3.5	0	6	28	11	28	604	120	17	6	4	3	11	3	3	4	16
386	4	8.7	0	9	240	97	29	1520	662	30	6	11	29	52	2	3	4	21
410	4	6.5	0	9	500	271	35	4837	1403	22	75	47	81	199	25	39	73	66
387	5	10.2	0	8	25	10	29	24	14	37	4	0	0	12	0	0	0	0
423	3	3.5	10	6	28	193	87	2100	2783	57	55	2	1	9	23	1	0	1
427	3	3.5	25	6	49	285	85	475	2339	83	83	28	16	1	14	20	15	4
429	3	3.5	25	6	44	135	75	4586	4151	48	44	24	21	30	4	7	5	3
384	4	7.4	5.5	9	330	465	58	1851	2741	60	75	58	53	78	9	17	10	12
407	5	4.7	5.5	8	31	123	80	43	72	63	35	3	0	7	22	2	2	7
408 ¶	5	4.7	5.5	5	<2	29	>93	0	0		46	0	0	6	12	0	0	0

* Not listed are two recipients in experimental group that died before being killed and one member of control group and two of experimental group that were negative for donor cells and Ig's. † Cells from donor 3 were incubated for 24 hours; those from donors 4 and 5 were incubated for 18 hours. ‡ Abbreviations are: WBC, leukocytes; Ig's, immunoglobulins. § Total cells counted are given. Counts from recipients of donor 3 are approximately per 2×10^5 WBC. ‖ Total clusters on three whole transverse sections are given. Size refers to number of cell profiles in section through cluster. ¶ Blood and tissues obtained immediately after spontaneous death.

of recipients on the basis of the predominant allotype marker of most clusters and the sizes of these clusters.

Clusters of immunocytes in the repopulated spleen arise as a result of cell proliferation; this is clear since the number of cells containing donor allotype in a recipient's spleen alone often exceeds the total number of transferred leukocytes (*4*). It is not known whether the precursors of these clusters are already committed to giving rise to daughters that produce only one parental allotype or the other. If the precursors are already committed before culture in vitro, the apparent stimulation of proliferation of b9 cells by exposure to antibody to b9 may be due to enhanced survival of their precursors before transfer, to an absolute increase in the number of such precursors during culture, or to an earlier initiation of proliferation and differentiation of b9 cells compared with b5 cells. It is known that antibodies to one parental allotype stimulate only a fraction of rabbit peripheral blood lymphocytes from heterozygotes to transform into blast cells (*9*). If the precursors of clusters formed in recipients' spleens are among those cells transformed, it may be that the action of antibodies to b9 is to initiate the proliferation and differentiation processes of appropriately committed cells in vitro before transfer.

Whether the transfer into an in vivo milieu allows the visualization of the eventual outcome of blast transformation of some cells in vitro is still unknown. Since discrete clusters of immunocytes can contain mixtures of cells making antibodies of different specificities, it is likely that they do not represent clones (*4*). The relative homogeneity of most of the donor cell clusters in both the control and experimental groups of recipients with respect to cells making one or the other parental marker suggests that some type of selective aggregation may occur in vivo after transfer or even before, during the in vitro culture period. The opposite presumption, that precursors may be uncommitted with respect to the allotype that their daughters will express, would change the interpretation of much of the above. Precursors may be influenced by antibody to b9 to give rise to daughters that produce b9 rather than b5 molecules. This mechanism should result in fewer b5 cells and molecules in recipients receiving treated cells compared with controls, but we have not found such a trend. The observations reported here may provide an insight into the mechanism of "allotype suppression" (*10*) which is the alteration in vivo of allelic expression resulting from the exposure of neo- or prenatal rabbits to antibodies to the corresponding allotype. Moreover the system may offer a practical approach to the selective expansion of immunocytes making a particular class, type, allotype, or idiotype of immunoglobulin from a mixed population of precursor cells.

ASHER FRENSDORFF
PATRICIA P. JONES
YOHEVED BERWALD-NETTER
JOHN J. CEBRA
Department of Biology, Johns Hopkins University, Baltimore, Maryland 21218
ROSE MAGE
Laboratory of Immunology, National Institute of Allergy and Infectious Diseases, Bethesda, Maryland 20014

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Epithelial Origin of Polyoma Salivary Tumors in Mice: Evidence Based on Chromosome-Marked Cells

Abstract. *Trypsin dissection of epithelium from mesenchyme of salivary gland rudiments allows reassembly of glands having either epithelium or mesenchyme selectively marked by T_6 chromosomes. Virus-induced tumors in such glands invariably bear the karyotype of the epithelial component. The method solves a specific example from a group of classical problems concerning epithelial as opposed to mesenchymal origin of neoplasms.*

Identification of tissue and cell type of origin of neoplasms has traditionally depended on recognition of one or more phenotypic characters of the neoplastic cells. These characters may be morphological, biochemical, or functional. For example, an islet cell tumor of pancreas may be characterized in part by the resemblance of its cells to β cells, by the demonstration of insulin production by the tumor, or by manifestations of hyperinsulinism in the host.

When specific characters are lacking, or when those available conflict with one another in nosologic significance, other methods of identification must be devised. We report here a method in which a karyotypic marker is used for the identification of the tissue type of origin of a problematic neoplastic group, that is, the polyoma virus–induced neoplasms of mouse submandibular gland.

The method (Fig. 1) combines use of the T_6 chromosome–marker system (*1*) with the trypsin technique (*2*) for separating epithelium from mesenchyme of gland rudiments. Given these laboratory devices, one can dissect apart the two tissue types of salivary gland rudiments and reassemble them so that either the epithelial or the mesenchymal component is karyotypically marked. This manipulation creates an exception to one of the limitations cited by Ford (*1*) in delineating the uses of chromosome markers: ". . . present methods of preparation do not permit the assignation of mitotic cells to different histological or hematological classes." Blastomeric fusion as performed by Mintz (*3*) offers another way to obtain tissues and organs with mixed karyotype, but does not permit precisely controlled segregation of a karyotypic marker within mesenchyme or epithelium of salivary rudiments.

Polyoma-free mice of strains C_3H/Bi and $CBA/H\text{-}T_6T_6$ were bred in our isolated colonies. Embryos were taken at 13 and 14 days of gestation, to supply submandibular gland rudiments at stages 2 to 5 of Borghese (*4*). Rudiments excised from embryos of both strains on the same morning were separately dis-