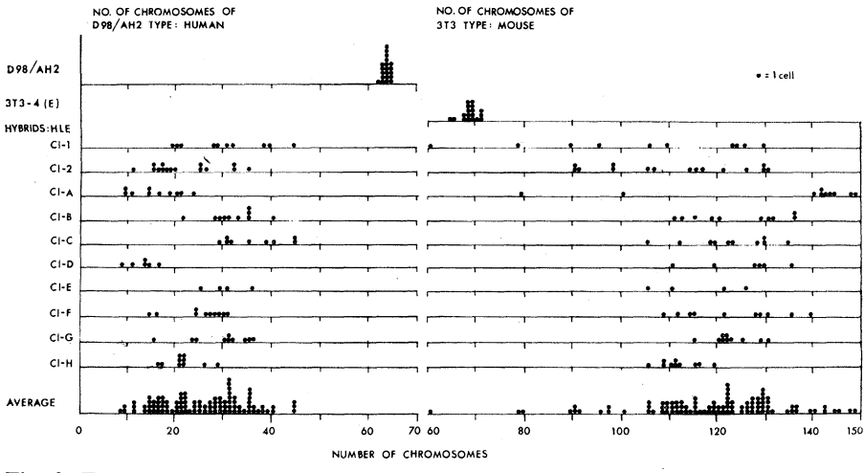

Fig. 2. Frequency distribution diagram of human type and mouse type chromosomes in ten clones of human-mouse hybrid HLE.

per cell is therefore considerably higher than in the hybrids described previously (*2*, *4*); but because of the duplication of the 3T3 complement, an unusual feature of this combination, the ratio of human to mouse genetic material is still only about 1:4. A karyotype of HLE-cl-C is shown in Fig. 1C; 38 chromosomes have been classified as human, and among them are members of every group.

The hybrid cells were very large. Growth was very slow at first, but within 2 months growth rates of the hybrids were of the same order as those of the parent lines, or slightly lower. On serial culture in standard medium, there was a slight tendency toward further loss of human chromsomes; clone C decreased in modal human chromosome number from 38 to 17 over the course of 100 cell generations. There was no concurrent loss of mouse chromosomes. Preferential loss of human chromosomes therefore occurs as in the human-mouse hybrids reported earlier (*2*, *4*), but considerably more slowly.

In an attempt to introduce a greater number of human chromosomes, a hybrid line was rehybridized in the following manner. A clone of HLE-C was transferred into medium containing BUDR at a concentration of 30 μg/ml. This selectively eliminates cells with the human chromosome bearing the thymidine kinase gene (*2*, *3*). Approximately 1 percent of the cells survived and grew into TK$^-$ colonies. One subline originating in this way had an average of 11 human chromosomes per cell and an undiminished number of 3T3 chromosomes. This population was mixed with D98/AH2 and the hybridization carried out in the usual manner, with the use of HAT to eliminate the parent cell types. Four clones of hybrid cells were isolated. All possessed from 15 to 45 human chromosomes, clone AB2 having the highest mode of 34 chromosomes. The number of chromosomes per cell was therefore not higher than that obtained after a single hybridization (HLE-C had a modal human chromosome number of 35).

Thus the selective elimination of human chromosomes occurring in human-mouse somatic cell hybrids reflects fundamental properties relative to the two species, while the culture history of the parent lines plays only a small part. To date the highest numbers of human chromosomes per hybrid cell occur in the HLE hybrids. Since (i) chromosome loss appears random, (ii) the average number of human chromosomes in some clones considerably exceeds 23, the number of haploid human chromosomes, and since most parent cell functions are expressed in somatic cell hybrids, most human gene products found in the human parent cell may be expected to be present in the HLE hybrids. For example, in contrast to the human-mouse hybrids reported earlier (*2*), which lack human gene products necessary for polio virus infection, the HLE hybrids are susceptible to this virus and may be used to identify human chromosomes bearing cellular genes necessary for the infectious process. In addition, study of these hybrids has revealed an interesting problem with respect to the control of ribosomal RNA synthesis, which will be reported elsewhere.

YUTAKA MATSUYA*
HOWARD GREEN
Department of Cell Biology, New York University School of Medicine, New York 10016

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* Present address: Research Institute for Tuberculosis, Leprosy, and Cancer, Tohoku University, Sendai, Japan.

7 November 1968

Human Serum Inhibitor of C'1 Esterase: Identity with α_2-Neuraminoglycoprotein

Abstract. *A comparison of highly purified C'1 esterase inhibitor from human serum and α_2-neuraminoglycoprotein from human plasma by immunofusion, immunoelectrophoresis, and discgel electrophoresis showed them to be antigenically identical.*

The esterase activity (C'1 esterase) associated with the first component of human complement can be blocked by a protein inhibitor in normal human serum (*1*). This inhibitor (EI) is a heat- and acid-labile α_2-globulin which com-

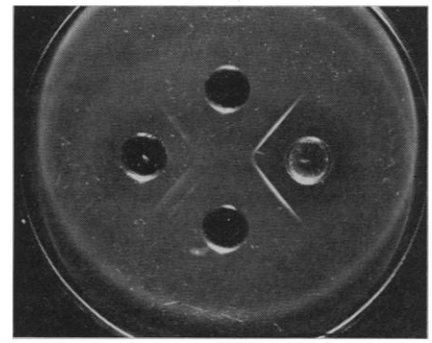

Fig. 1. Ouchterlony double diffusion experiment. Wells, cut in 0.5 percent (weight to volume) agarose, contained: top, C'1 esterase inhibitor; bottom, α_2-neuraminoglycoprotein; right, antiserum to C'1 esterase inhibitor; left, antiserum to whole human serum. Photographed after 26 hours of diffusion at room temperature.

bines rapidly and stoichiometrically with purified C'1 esterase (*2*, *3*). The highly purified EI contains about 12 percent hexose and 17 percent *N*-acetylneuraminic acid (*4*). These properties, together with its sedimentation constant and absorbancy ($E^{1\%}_{1cm}$) were strikingly similar to those of α_2-neuraminoglycoprotein (α_2NGP) isolated by Schultze *et al.* from human plasma (*5*). This report presents immunologic and electrophoretic data which indicate that this inhibitor and the glycoprotein are identical.

The esterase inhibitor was isolated from serum in highly purified form (*6*), and α_2NGP was purified from human plasma (*5*). The α_2NGP preparation was 90 to 95 percent pure and contained a small amount of orosomucoid. Each protein was dissolved (4 mg/ml) in 0.005*M* sodium phosphate buffer, *p*H 6.7. Rabbit antiserum to EI was prepared by repeated injections of purified EI into rabbits; the antiserum was made monospecific by absorption with serum from a patient with hereditary angioneurotic edema, a disease in which there is little or no circulating EI (*7*). Horse antiserum to whole human serum was obtained from a commercial source.

The esterase inhibitor and α_2NGP reacted with rabbit antiserum to EI to form a line of complete identity (Fig. 1). A line of identity was also found with the horse antiserum to human serum. In addition, a second line was formed between α_2NGP and horse antiserum to human serum, indicating a small amount of impurity (orosomucoid) in the former.

Immunoelectrophoresis (*8*) of EI and α_2NGP against rabbit antiserum to EI and horse antiserum to whole serum is shown in Fig. 2. Both EI and α_2NGP produced a single arc in the α_2 position against monospecific rabbit antiserum to EI (Fig. 2a) and horse antiserum to whole human serum (Fig. 2b); further, a mixture of equal volumes of EI and α_2NGP also produced a single, somewhat less symmetrical arc in the same position (Fig. 2c). The EI concentration in normal serum is probably too low to form a precipitate arc against the antiserum used (Fig. 2a).

A comparison of the two protein preparations by analytical disc-gel electrophoresis (*9*) showed that, in 10 percent acrylamide gel, both EI and α_2GNP produced a similar broad band relatively close to the top of the separating gel; the presence of a trace amount of orosomucoid in the α_2NGP preparation was demonstrable as a faint, more anodic band. The broadness of the major bands increased in gels of lower acrylamide concentration.

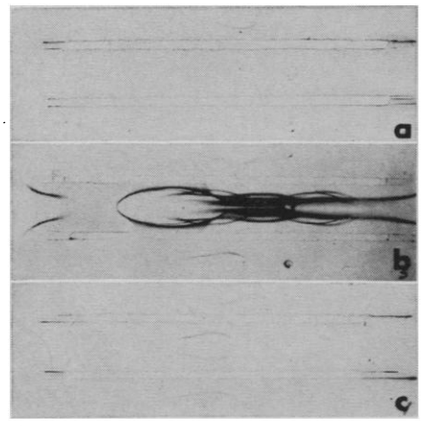

Fig. 2. Immunoelectrophoresis. (a) Top well, C'1 esterase inhibitor; center well, normal human serum; bottom well, α_2-neuraminoglycoprotein. Troughs contain rabbit antiserum to C'1 esterase inhibitor. (b) Top well, C'1 esterase inhibitor; center well, normal human serum; bottom well, α_2-neuraminoglycoprotein. Troughs contain horse antiserum to normal human serum. (c) Top well, C'1 esterase inhibitor; bottom well, α_2-neuraminoglycoprotein; center well, mixture of equal volumes of samples used in top and bottom wells. Troughs contain rabbit antiserum to C'1 esterase inhibitor. The anode is to the left.

Although a more rigorous chemical comparison is lacking, it appears from the immunochemical evidence that EI and α_2NGP are the same protein. The preparation of α_2NGP was inactive in inhibiting C'1 esterase because of the acid conditions used in its isolation; the ability of EI to inhibit C'1 esterase is also irreversibly destroyed below *p*H 5.5 (*2*).

JACK PENSKY

Institute of Pathology,
Case Western Reserve University,
Cleveland, Ohio 44106

H. GERHARD SCHWICK

Behringwerke AG,
Marburg/Lahn, West Germany

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30 October 1968

Mechanism and Prevention of Fixed High Vascular Resistance in Autografted and Allografted Lungs

Abstract. *The fixed vascular resistance observed in transplanted lungs and attributed to denervation can be avoided by angioplastic widening of the pulmonary artery anastomosis. With distensible arterial anastomoses, autografted and allografted lungs vasodilate normally with increased flow and can sustain dogs whose contralateral pulmonary artery is ligated immediately after lung transplantation.*

Transplantation of a lung with simultaneous ligation of the opposite pulmonary artery has caused death of the recipient animal within a few days (*1*). Death has been attributed to fixed, increased intrinsic vascular resistance in the transplanted lung regarded as consequent to its denervation (*1*, *2*). Forcing the transplant to accept the entire pulmonary blood flow has been thought to cause either fatal right heart failure or pulmonary insufficiency secondary to damage to the pulmonary microvasculature. In view of the fact that human recipients of lung transplants will have vascular disease in the remaining lung, the ability of laboratory animals to survive transplantation of one lung and simultaneous ligation of the opposite pulmonary artery is important in determining the feasibility of therapeutic lung transplantation.

We studied five groups of six dogs each, before and after the right pulmonary artery (RPA) was ligated through a left thoracotomy. Group 1 comprised normal control animals. In animals of group 2, a snug-fitting but not constricting cloth band was placed around the left pulmonary artery (LPA) before ligation of the RPA. In animals of group 3, the LPA, the left mainstem bronchus, and a cuff of the left atrium containing the left pulmonary veins were transected. The lung was then removed from the chest, perfused with 300 ml of cold (4°C) Ringer's lactate solution (U.S.P.) at a pressure of 25 cm-H_2O, and replaced in the chest. Vascular and bronchial continuity was restored with fine, continuous, braided dacron sutures. Then the RPA was ligated. Lungs were ischemic for 50 to 70 minutes. In animals of group 4, the left lungs were removed and replaced like those in group 3 except that the diameter of the pulmonary artery at the site of suture was increased by a patch of jugular vein