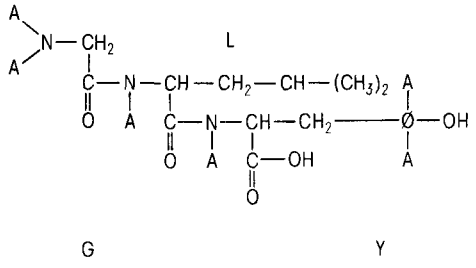
activity possible would be 6 moles TCEA per mole peptide ('A' standing for alkylans):



Alkylation products were characterized by radioactivity, by absorption at 280 nm, and by spot tests[4,5]. Besides monoalkylation, cyclization, and/or cross-linkage of the peptide molecule(s) with the trifunctional alkylans can occur. (Alternatively, alkylation of Y at the phenylic OH-group cannot be excluded.)

Chromatography. When the incubation mixture was chromatographed on silica gel TLC plates spots of radioactivity were found which did not give a ninhydrin reaction[2] but were clearly visible under UV-light. Some alkylation products were isolated from the incubation mixture by gel filtration on a column of BIOGEL P-2 (1 × 145 cm; in 0.1 M NH_4HCO_3). After determining radioactivity and absorption at 280 nm in the eluted peaks sp. act. of 1, 1.5, 2, and 4 moles TCEA bound to 1 mole of peptide were calculated. Only products with specific activities less than 2 showed a slight color after treatment with tert-butylhypochlorite and starch iodine solution using the method of Mazur et al.[5].

Hydrolysis and amino acid analysis. As judged from the TL chromatograms of the original peptide and of the incubation mixture no splitting of the peptide into G, L, and Y, or G and L-Y, or G-L and Y occurred during alkylation and incubation, respectively. Free (alkylated) amino acids were obtained, however, by hydrolysis with 6 N HCl (under N_2 at 110 °C for 22 h) or with 6 N NaOH (under N_2 at 110 °C for 22 h) or with carboxypeptidase A (twice washed CP-A in 0.2 M $NaHCO_3$, pH 9.3 at 37 °C for 22 h). From a hydrolyzed alkylated peptide with a sp.act. of 1.5 (TCEA:peptide) L and Y could easily be detected with an automatic amino acid analyzer. G, however, gave no or only a very poor ninhydrin reaction. The amount of ninhydrin positive G, L, and Y decreased with increasing specific activity of the hydrolyzed peptide. None of the ninhydrin positive amino acid peaks, eluted from the ion exchanger of the amino acid analyzer, contained any radioactivity. The radioactivity was eluted with a LiOH solution which is generally used for regeneration of the column. When this eluate was filtered on a column of BIOGEL P-2, up to 6 radioactive peaks could be distinguished.

Autoradiography. TLC of the above hydrolysates of alkylated peptides did not yield a ninhydrin positive G spot. The higher the specific activity of the hydrolyzed peptide, the less was the color of the corresponding spot for G, L, and Y on the TL plate after development with ninhydrin. Autoradiography, however, clearly yielded radioactive spots on the TL chromatogram for all 3 amino acids. Their R_f-values were slightly different from those of free (not alkylated) G, L, and Y. Free G, L, or Y were not readily alkylated by TCEA under same conditions as described for the peptide. Although this is not explicable an even better accessibility for alkylation of the amino acid and/or of the peptide bond in the peptic compound may be indicated. Perhaps a more distinct polarity in the tripeptide favors a better reactivity of the terminal NH_2-group with the alkylans.

Ross[6] did not mention a reaction of protein, peptides or even Y with TCEA. Our results could be interpreted by suggesting that TCEA alkylates protein (and peptides) at the nitrogen of a free NH_2 group and/or at the nitrogen of the peptide bond as well. The unspecific ability of protein, previously treated with p-chloromercuribenzoate, N-ethylmaleimide, or diethylpyrocarbonate[2], to bind TCEA might support this idea. The lack of ninhydrin reaction and the failure to detect a H-N-bond[5] in the alkylated peptide seems to be due to its alkylation. The accessibility of a peptide bond to the alkylans TCEA obviously depends on the steric structure of the protein molecule.

The binding of the alkylans TCEA to the protein seems to be stronger than the peptide bond as judged from the hydrolysis experiment (with HCl or NaOH). Furthermore, the alkylation of the peptide bond seems to be no steric restriction for the enzymatic cleavage of the peptide with carboxypeptidase A or trypsin[2]. This fact might be an advantage for alkylation repair in the living cell.

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0014-4754/83/030304-02$1.50 + 0.20/0

Detection of factor VIII related antigens in long term cultures of rat endothelial cells [1]

A.T. Darnule, G. Stotzky, T.V. Darnule, M.S. Parshley, G.M. Turino and I. Mandl

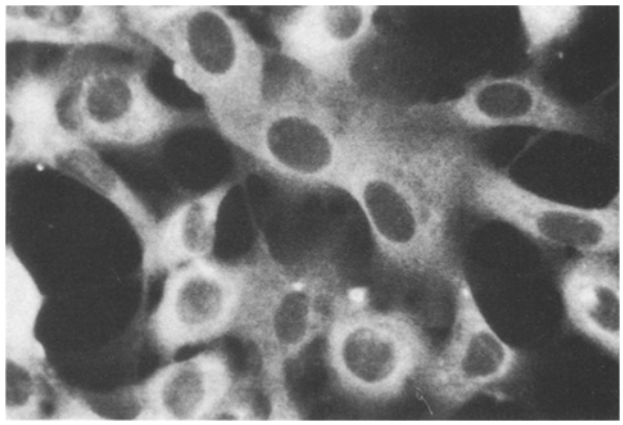
Columbia University, College of Physicians and Surgeons, 630 West 168th Street, New York (New York 10032, USA), and New York University, Department of Biology, New York (New York 10003, USA), July 20, 1982

Summary. Rat endothelial cells in culture can be distinguished from fibroblasts and epithelial cells by their reaction with antisera against human factor VIII (AHF) associated proteins.

Endothelial cells can be identified in vivo and in culture by their morphological characteristics and by their ability to synthesize angiotensin converting enzyme[2-4] and antihemophilic factor VIII[5,6]. We have previously reported[7] the establishment of a strain of cells derived from a single cell cloned by the microplate method from a line of mixed

normal adult rat lung cells. On the basis of their morphological and growth characteristics and their ability to synthesize angiotensin converting enzyme the cells have been identified as endothelial cells. We have also shown that they synthesize glycosaminoglycans[7] and more recently have demonstrated that they produce crosslinked mature elastin[8]. In addition, we have detected surface antigens which appear to be specific for endothelial cells amongst the antigenic determinants of this cell line[9]. Further support for its identification as an endothelial cell became therefore of considerable interest. Blood coagulation factor VIII synthesis has been considered one of the most important criteria for the identification of endothelial cells in vivo and in culture. Monospecific antibodies to antihemophilic factor VIII derived from human plasma and bovine plasma have been used to identify human and bovine endothelial cells, respectively[6,10]. Previous attempts in our laboratory to produce anti-rat factor VIII were unsuccessful because adequate volumes of rat blood were not available for isolation of the antigen. Recently Schwartz[11] has shown that anti-human factor VIII crossreacts with bovine endothelial cells indicating that species specificity if it exists at all is not absolute. The present study was undertaken to determine whether anti-human factor VIII would also crossreact with rat lung cells and could be applied to support their identification as endothelial cells.

Coverslip cultures of the rat lung cells were incubated with rabbit antibodies to human factor VIII and associated proteins (Calbiochem-Behring, San Diego, CA) and fluorescein-conjugated goat anti-gamma globulin. They were then examined by indirect immunofluorescence microscopy. The figure shows a granular yellowish green fluorescence localized around the nucleus in the cytoplasm with little or no fluorescence in the nucleus. Fibroblast and epithelial cells derived from normal rat kidney (American Type Culture Collection No.CRL1570 and 1571) were tested as controls and showed no or little fluorescence indicative of factor VIII synthesis. Similarly rat lung cells incubated with normal rabbit serum produced no fluorescence beyond the very low level nonspecific background exhibited by fluorescein conjugated goat anti-gamma globulin. Results obtained confirm our previous identification of the rat lung cells. In addition, they demonstrate that although the clone of endothelial cells has been in culture for numerous passages, it has retained not only its morphological characteristics but also its capacity to synthesize factor VIII antigen.



Fluorescence staining of rat lung endothelial cells by rabbit anti-human factor VIII serum (dilution 1:8). × 125.

1 The data form part of the Ph.D. thesis submitted by A.T. Darnule to the Department of Biology, New York University. Supported in part by NIH USPHS Program Project grant HL15832.
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0014-4754/83/030305-02$1.50+0.20/0

Quantitation of the soluble receptor of human T lymphocytes for sheep erythrocytes by electroimmunodiffusion in the serum of patients with cancer, uremia and leprosy[1]

N.C. Moura, I.M. Longo, L.A.G. Bernd and N.F. Mendes

Disciplina de Imunologia, Escola Paulista de Medicina, Rua Botucatu, 862, C.P. 20300 Agencia Vila Clementino, São Paulo (São Paulo, Brasil), June 28, 1982

Summary. Abnormally high serum levels of the soluble receptor of human T lymphocytes for sheep erythrocytes were found, by electroimmunodiffusion, in patients with carcinoma or other solid tumors, leukemia, lymphoma, uremia and lepromatous leprosy.

Human T lymphocytes carry a membrane receptor for sheep erythrocytes[2-5] which can be recovered in a soluble form (R_s) from the supernatant of heated (45 °C) peripheral lymphocytes[6]. We have obtained a specific anti-receptor serum (anti-R_s) by immunizing sheep with autologous erythrocytes (E) sensitized by R_s. This antiserum is cytotoxic for T cells, inhibits E-rosette formation, agglutinates ER_s complexes and can be used to identify T lymphocytes by immunofluorescence[7]. In this study, we have investigated the ability of the anti-R_s serum to detect R_s in human