Advances in CELL CULTURE

VOLUME 5

Cell Separation

METHODS AND SELECTED APPLICATIONS

EDITED BY

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Preface

In 1975, we published a general review of methods of cell separation. Because of the interest in this review, we planned a sabbatical year to write a book with the same scope. Between the writing of the first review (1973–1974) and the attempt to write a book (1977–1978), the references to be cited increased from somewhat more than five hundred to somewhat more than seven thousand. Our bibliography pertinent to this methodology was expanding at a rate of two to four dozen articles weekly, and we were compelled to face the fact that it was no longer feasible for one or two authors to address this area adequately. The rapid growth in this area led us to plan this multivolume, multiauthored treatise.

In approaching this work, it was our goal to select critical authors with considerable personal familiarity with the design and/or application of methods for the separation of cells. Rather than attempt comprehensive reviews, they were asked to address relatively finite subjects and to include sufficient references to direct those readers who want more information to the appropriate sources. We have attempted to address this work to a heterogeneous audience of experimental oncologists, hematologists, immunologists, cell biologists, endocrinologists, and others who are not already expert in the use of methods for cell separation. We are grateful that most of those invited to contribute to this work found the time to do so, and we hope that their critical, quantitative approaches to problems in cell separation will stimulate new investigators to examine critically many of the "accepted" methods for cell separation.

THOMAS G. PRETLOW II
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Chapter 1

Isolation and Characterization of Human Vascular Endothelial Cells with Application to Studies of the Subendothelial Matrix

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I. Introduction

The vascular endothelium is a crucial continuous monolayer that lines all blood vessels, including the conducting vessels (such as the aorta) and the microvasculature (composed of arterioles, capillaries, and venules). This endothelial cell barrier provides a nonthrombogenic interface with the blood and acts as an efficient conduit by which nutrients, metabolic wastes, hormones, and blood cells are rapidly transported throughout the organism. Besides providing a semipermeable membrane, the endothelium participates in wound healing and neovascularization and plays an important role in the pathogenesis of many disease states, including atherosclerosis, thrombosis, and tumor progression and metastasis, to name a few. The extreme diversity of endothelia is only now becoming apparent as the various ultrastructural, physiological, and biochemical aspects of the vasculature are being uncovered. Much of the newly acquired infor-

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mation concerning the functions of this complex organ system is a direct consequence of the successful isolation and culture of the endothelial cells lining the blood vessel.

The endothelial cell is fastidious and requires a number of unique conditions for its survival and continued proliferation. Furthermore, the environmental requirements for each type of endothelial cell may vary substantially, even in the same species. The realization that most endothelial cells require highly specific peptide growth factors for optimal proliferation and the availability of these growth factors have accelerated advances in this field. Another hurdle that had to be overcome was that while certain endothelia, such as the large-vessel cells, are easily isolated free of other stromal cell types, such as fibroblasts or smooth muscle cells, the microvascular endothelial cells are usually enmeshed in a connective tissue that contains other cells, making their selective removal difficult. Consequently, specialized techniques had to be developed to permit clean isolation of the endothelial cells. Obviously, human tissue is the most relevant source for studies of endothelial cell biology and disease states. Fortunately, not only can both macrovessel and microvessel endothelial cells be successfully isolated and serially passaged, but in addition, these cultured cells seem to retain many of their differentiated functions.

The production of a subendothelial basement membrane matrix is a unique feature of most blood vascular endothelial cells. However, this subendothelial matrix may vary according to location and species. Certain highly specialized vascular endothelia may lack a distinct basal lamina, as is the case with the liver sinusoid endothelium. Elsewhere, the basement membrane may be fused with matrix of neighboring epithelial cells, such as the capillary endothelia present in the glomerulus and lung alveoli. In most cases, the basement membrane matrix acts as a permeability barrier, as well as a scaffolding to which the endothelial cell forms its polarized attachment. In the case of the microvasculature, this structure may provide flexibility but also integrity to the vessel (Murphy and Johnson, 1975). During neovascularization, one of the earliest events is the penetration of the microvascular basement membrane by endothelial cells and their invasion of the surrounding perivascular connective tissue matrix (Ausprunk and Folkman, 1977). The basal lamina is probably important during the regeneration of damaged vessels, acting as an insoluble pathway by which endothelial cells migrate into the injured tissue to reform new capillary loops. Finally, information obtained from culture of endothelial cells has suggested that their continued proliferation and maintenance of their unique phenotype is dependent on the macromolecular composition of the substrate to which they adhere (Gospodarowicz, 1983).