Fundamentals of Human Lymphoid Cell Culture

J. LESLIE GLICK

Fundamentals of Human Lymphoid Cell Culture

J. LESLIE GLICK
Genex Corporation
Rockville, Maryland

MARCEL DEKKER, INC. New York • Basel

Library of Congress Cataloging in Publication Data

Glick, J Leslie, [date]
Fundamentals of human lymphoid cell culture.

Includes bibliographical references and index.
1. Human cell culture. 2. Lymphocytes.
I. Title.
QH585.G48 611'.0185 80-15725
ISBN 0-8247-6988-0

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

Current printing (last digit):
10 9 8 7 6 5 4 3 2 1

PRINTED IN THE UNITED STATES OF AMERICA

Foreword

It is a pleasure to introduce *Fundamentals of Human Lymphoid Cell Culture,* by Dr. J. Leslie Glick, to the community of scientists in cell culture. The book's usefulness lies primarily in the culture of lymphoblast cell lines, but the reader will find applications of principles and practice to many other cell lines and cell culture problems, particularly in immunology, genetics, virology, and molecular biology.

In 1966, Les Glick was at Roswell Park Memorial Institute and was part of a group of scientists working with George E. Moore and Jun Minowada on the establishment of animal and human leukemic cell lines. Their subsequent establishment of lines from healthy blood donors and the contributions of Phil Glade, Kurt Hirschorn, and Art Bloom at other institutions led to the propagation of thousands of lymphoblast lines for research purposes. Since Moore and his associates first began establishing lines from peripheral blood lymphocytes, they are now available from hundreds of individuals representing leukemias, genetic deficiency diseases, and most HL-A haplotypes. This remarkable genetic library is now, for the most part, on file at the American Type Culture Collection, Rockville, Maryland, and at the Institute for Medical Research, Camden, New Jersey.

This book does not portend to be a comprehensive review of the topics covered, but is rather a carefully worked out combination of background, theory, and application. The practical laboratory section in each chapter contains "hands on" technical lore gleaned from research publications and has evolved from several years of teaching a successful course in lymphoid cell culture while the author was at the head of Associated Biomedic Systems, Inc. in Buffalo. For example, Chapter 2, "Cell Culture Environment," contains information on pH control and water preparation not heretofore collected in one place. Sterile technique, record keeping and maintenance, mycoplasma control, and cryogenic storage comprise the

elements of day-to-day culture upkeep and are presented didactically and as "case method" problems for analysis. A final section on biological characterization is again treated by the case method and presents principles of cross-contamination detection. Cell identification techniques are described which include: chromosome preparation, HL-A typing, EBV virus detection, rosette formation and surface antigens. Thus, even though we can all benefit from the background sections of each chapter, the place for the book is at the bench where investigators and technicians will find ready answers to many of their laboratory problems involving lymphocyte cultures in one volume.

Ben W. Papermaster, Ph.D.
Scientific Director
Cancer Research Center
Columbia, Missouri

Preface

This book is the natural outgrowth of a series of workshops which my colleagues and I first organized in 1973 at Associated Biomedic Systems in Buffalo, New York. The workshops were designed to teach the biomedical researcher how to manipulate cultured human lymphoid cells. We found out very quickly that individuals of diverse backgrounds–M.D.'s, Ph.D.'s, technicians, and graduate students–were interested in learning about the cultured lymphocyte.

Human lymphoid cell lines are readily established from the peripheral blood. They propagate indefinitely in large-scale culture units. They have relatively short population-doubling times. They continue to synthesize in vitro a number of unique cell products which are characteristic of lymphocytes in vivo. As a result, human lymphoid cell technology is utilized in virtually all the major biomedical research disciplines, including biochemistry, genetics, immunology, pharmacology, physiology, and virology.

The number of investigators who work with human lymphoid cell lines has grown very rapidly in the past few years. Researchers who have had little or no experience in tissue culture want to handle the cultured lymphocyte. Most of these individuals have had only a sketchy background in hematology. This burgeoning group is to whom this book is addressed.

The book is actually a manual. It is divided into three parts. The first part offers an overview of the field. The other two parts deal with culture techniques and biological assays. Cookbook presentations abound here. My intention is to make the technology understandable to practically anyone who wishes to begin applying it.

I am indebted to my colleagues, Ann Dutton, Leonard Borzynski, and Alex Burns, for their advice and assistance in preparing this manual. Much of the technology described in the manual has been advanced due to their efforts.

I also want to acknowledge a very fruitful collaboration with Ben Papermaster. In 1969 we formed Associated Biomedic Systems for the purpose of developing applications involving the cultured lymphocyte. Finally, I owe a debt of gratitude to George Moore, the founder of human lymphoid cell technology. Almost 15 years ago at Roswell Park Memorial Institute in Buffalo, New York he opened my eyes to both the realities and the potentialities of this exciting technology.

J. Leslie Glick

Contents

part I

Overview

chapter 1

Biological Properties of Established Cell Lines

A Brief History

A major objective of hemopoiesis research is to enable human bone marrow and peripheral blood cells to proliferate in long-term culture in a highly differentiated state. Much progress has been made since 1964, when Iwakata and Grace [1] first established a cultured lymphocyte-like cell line from human peripheral blood. The cell line, designated RPMI 6410, originated from a patient with acute myeolgenous leukemia.

It was actually the extraordinary patience of Dr. Iwakata, who was then a visiting fellow in Dr. Grace's laboratory at Roswell Park Memorial Institute (RPMI) in Buffalo, New York, that launched the field of human lymphocyte cell culture. Iwakata did not discard what would have appeared to others to be a moribund culture. Instead, he maintained the patient's leukocytes in culture medium for 1½ months before the onset of cell proliferation.

Dr. George E. Moore, who at the time was Director of RPMI, immediately began a crash program to establish hundreds of different human lymphocyte-like cell lines in long-term culture [2, 3]. Such cell lines are now known as lymphoid or lymphoblastoid cell lines. Moore is personally responsible for the plurality of lymphoid cell lines used throughout the world today. In 1967 Moore, Gerner, and Franklin [4] first reported success in establishing human lymphoid cell lines from the peripheral blood of normal donors. Moore's laboratory also first devised means of growing lymphoid cell lines in both continuous-flow and batch-suspension culture units in quantities ranging from 10 ml to over 1000 liters per culture vessel [3, 5-7].

Coincidentally, in 1964, the same year that Iwakata and Grace reported their discovery, Pulvertaft [8] and Epstein and Barr [9] independently reported

the establishment of Burkitt lymphoma cell lines. Although the Burkitt lines were derived from biopsy tissue, they had much in common with the lymphoid cell lines derived from peripheral blood at RPMI. As was subsequently discovered, the vast majority of human lymphoid cell lines, whether derived from Burkitt lymphoma patients, leukemia patients, or normal donors, possess properties characteristic of B cells, i.e., bone marrow-derived lymphocytes. Furthermore, almost all human B-cell lines harbor the Epstein-Barr virus (EBV) genome. This virus has not only been implicated in the etiology of Burkitt lymphoma, but has also been reported to cause infectious mononucleosis. The peculiar relationship between EBV and lymphoid cell lines is discussed further on.

Although most human lymphoid cell lines are representative of B cells, a few of these lines have been characterized as T cells, i.e., thymus-derived lymphocytes. In 1965 Foley et al. [10] established the first human T-cell line from the peripheral blood of a patient with acute lymphoblastic leukemia. However, it was not until a number of years later that this cell line, CCRF-CEM, was actually characterized as a T-cell line. In 1972 Minowada, Ohnuma, and Moore [11] reported the MOLT cell lines as the first human T-cell lines so characterized. Like CCRF-CEM, MOLT-1, MOLT-2, MOLT-3, and MOLT-4 were also established from the peripheral blood of a patient with acute lymphoblastic leukemia.

More recently several lymphoid cell lines have been established with properties indicative of a third type of lymphocyte. Classified as null cell lines, these lines are all derived from acute lymphoblastic leukemia patients and possess neither B- nor T-cell markers [12-15].

Differentiation in Long-Term Culture

The remainder of this chapter highlights some of the characteristics of human lymphoid and related hemopoietic cell lines. Later chapters deal specifically with the methodologies involved.

Initiation and Establishment of Cultures

Establishment of continuous lymphoid cell lines from the buffy coat of peripheral blood is not as easy an accomplishment as the establishment of most other kinds of continuous cell lines from a variety of different tissues. First of all, the rate of success for establishment of human lymphoid cell lines generally varies from 5 to 50%, depending on the experience of the laboratory worker, the manner in which the blood is handled, and the size of the initial culture. Secondly, there is considerable delay in the length of time between initiation of the primary culture and establishment of the permanent cell line. This lag period averages 40 to 70 days [2], and while seldom less than 2 weeks, it may last as long

as 6 months. Finally, establishment of hemopoietic cell lines that constitute cell types other than B cells is a relatively rare event following the culture of human leukocytes.

Moore's technique for establishing lymphoid cell cultures was originally designed for processing large quantities of leukocytes from peripheral blood, which were obtained either with or without the aid of leukapheresis [2]. It was not unusual for him to initiate at the same time a dozen cultures from the buffy coat (leukocyte fraction) of a 500-ml collection of peripheral blood from a single donor. Each culture consisted of 50 ml of 5 to 15 million leukocytes/ml which were suspended in culture medium RPMI 1640 plus 20% fetal bovine serum (FBS). After 24 hr in culture, the number of viable cells dropped 80 to 90%. During the first week the culture was fed with 20% fresh medium. If during the second week the cell population fell below 0.5 million cells/ml, duplicate cultures were combined, and the culture medium was partially replaced 50 to 80% with fresh medium. In subsequent weeks the culture was fed with 10 to 20% fresh medium or centrifuged and fed with a combination of 80% culture supernatant and 20% fresh medium. The cell concentration was then adjusted to a final concentration of 0.5 to 1.0 million cells/ml. It was not unusual to continue the practice of combining duplicate cultures from the same donor in order to establish a cell line.

A lymphoid cell line is considered to be established upon the formation of free-floating cell clumps, a doubling of the cell population, and a decrease in the pH of the culture fluid. A truly established cell line is then capable of logarithmic growth of its cell population for an apparently unlimited number of generations.

A few years ago Glade and his colleagues [16, 17] developed a simple technique for establishing lymphoid cell lines from small aliquots of human blood. Phytohemagglutinin (PHA) was added to primary leukocyte cultures, each culture consisting of 10 ml of 1 to 3 million mononuclear cells/ml suspended in medium RPMI 1640 plus 20% FBS. Whereas cultures initiated from normal donors never appeared to become established without the addition of PHA, after the addition of PHA, 20 to 30% of the cultures initiated from normal donors did indeed become established after the usual latent period.

The most common procedure for inducing establishment of human lymphoid cell lines is to expose the primary cultures to EBV. This was independently discovered in three separate laboratories during the late 1960s [18-22], after it had become obvious that at least 25 to 50% of non-Burkitt lymphoid cell lines produced virus particles which appeared by means of electron microscopy to be identical to EBV [2, 4, 5]. Recent evidence indicates that lymphoid cell lines may become established within 2 weeks following exposure of only 0.1-ml aliquots of heparinized whole blood to filtrates of EBV-infected, B95-8 marmoset leukocyte cell cultures [23]. B95-8 cultures tend to release considerably greater

Table 1-1 Typical Example of the Combined Effect of Phytohemagglutinin (PHA) and Epstein-Barr Virus (EBV) on the Establishment of Human Lymphoid Cell Lines Originating from a Single Donor

Culture code[a]	Addition of substances[b]		Fate of culture		Designation of cell line
	Day 0	Day 3	Day 52	Day 150	
A	PHA	None	–	Dead	–
B	PHA	None	–	Dead	–
C	PHA	EBV	Established	Growing	ABS 0802
D	PHA	EBV	Established	Growing	ABS 0801
E	None	EBV	–	Dead	–
F	None	None	–	Dead	–

[a]Each culture (10 ml) consisted of 1 million leukocytes/ml suspended in a 25-cm^2 disposable plastic flask containing medium RPMI 1640 plus 20% fetal bovine serum. All cultures were initiated at the same time from the same sample of blood, which was drawn 12 hr previously.
[b]See Chapter 6 for details concerning PHA and EBV.

amounts of extracellular infectious EBV than most EBV-positive human lymphoid cell lines [24].

My laboratory has generated a procedure which combines both the PHA and EBV methods for establishing human lymphoid cell lines from small amounts of leukocytes. It is particularly useful when blood samples must be stored up to 12 hr prior to initiation of the primary cultures. Moore [3] had previously reported the requirement for initiating untreated primary cultures within the first 4 hr following collection of the blood, if the cultures were to become established. Our actual technique is described in Chapter 6, but Table 1-1 and Figure 1-1 illustrate the efficacy of combining the PHA and EBV procedures.

As demonstrated in Table 1-1, when 10-ml cultures were initiated from a sample of blood that had first been stored for 12 hr, only those cultures that were subsequently exposed to both PHA and EBV eventually resulted in established cell lines. Figure 1-1 shows the rate of cell proliferation of one of the newly established cell lines, ABS 0802. During an observation period of 26 days, the cell population doubled over 19 times. This represented a population-doubling time of about 32 hr. Subsequent analysis revealed that ABS 0802 was composed solely of B cells.

It is interesting that B-cell lines result following PHA treatment, since soluble PHA generally stimulates nucleic acid synthesis only in T cells. However, insoluble PHA, such as that bound to agarose beads, is capable of stimulating both B and T cells [25]. Presumably, the reason PHA is capable of a role in es-