Lymphokines 2

edited by

Edgar Pick

Maurice Landy

ADVISORY EDITOR

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Lymphokines

A Forum for Immunoregulatory Cell Products

EDITED BY

EDGAR PICK
Department of Human Microbiology
Sackler School of Medicine
Tel-Aviv University
Ramat-Aviv, Tel-Aviv, Israel

ADVISORY EDITOR
MAURICE LANDY
Schweizerisches Forschungsinstitut
Medizinische Abteilung
Davos, Switzerland

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EDITORIAL BOARD

List of Contributors

Numbers in parentheses indicate the pages on which the authors' contributions begin.

MIKE CECKA (125), *ICRF Tumour Immunology Unit, Department of Zoology, University College, London WC1E 6BT, England*

POJEN CHEN (273), *Department of Microbiology and Immunology, University of California, San Francisco, California 94143*

ALAIN L. DE WECK (1), *Institute of Clinical Immunology, Inselspital, Bern 3010, Switzerland*

ANN M. DVORAK (203), *Departments of Pathology, Beth Israel Hospital and Harvard Medical School, and the Charles A. Dana Research Institute, Beth Israel Hospital, Boston, Massachusetts 02215*

HAROLD F. DVORAK (203), *Departments of Pathology, Beth Israel Hospital and Harvard Medical School, and the Charles A. Dana Research Institute, Beth Israel Hospital, Boston, Massachusetts 02215*

VICTOR EDY* (1), *Theodor Kocher Institut, 3000 Bern 9, Switzerland*

PETER ERB (125), *Institute for Microbiology, University of Basel, Basel, Switzerland*

MARC FELDMANN (87, 125), *ICRF Tumour Immunology Unit, Department of Zoology, University College, London WC1E 6BT, England*

GEORGE E. GIFFORD (235), *Department of Immunology and Medical Microbiology, University of Florida College of Medicine, Gainesville, Florida 32610*

JOEL W. GOODMAN (273), *Department of Microbiology and Immunology, University of California, San Francisco, California 94143*

J. TERRELL HOFFELD (63), *Laboratory of Microbiology and Immunology, National Institute of Dental Research, National Institutes of Health, Bethesda, Maryland 20205*

* Present address: Flow Laboratories, Inc., 7655 Old Springhouse Road, McLean, Virginia 22102.

EVA MARIE KNIEP (1), *Max-Planck Institut für Immunbiologie, Freiburg, Federal Republic of Germany*

SIRKKA KONTIAINEN (87), *Department of Bacteriology and Immunology, University of Helsinki, 00290 Helsinki 29, Finland*

SANDOR LAZARY (1), *Institute of Clinical Immunology, Inselspital, Bern 3010, Switzerland*

GEORGE K. LEWIS (273), *Department of Microbiology and Immunology, University of California, San Francisco, California 94143*

DAVID Y. LIU (153), *Departments of Medicine, Harvard Medical School and the Robert B. Brigham Division of Brigham and Women's Hospital, Boston, Massachusetts 02115*

ZVI METZGER (63), *Laboratory of Microbiology and Immunology, National Institute of Dental Research, National Institutes of Health, Bethesda, Maryland 20205*

JUN MINOWADA (1), *Roswell Park Memorial Institute, Buffalo, New York 14263*

RUTH NETA (295), *Department of Microbiology, University of Notre Dame, Notre Dame, Indiana 46556*

RANKA ODAVIC (1), *Division of Hematology, Inselspital, Bern 3010, Switzerland*

JOOST J. OPPENHEIM (63), *Laboratory of Microbiology and Immunology, National Institute of Dental Research, National Institutes of Health, Bethesda, Maryland 20205*

NEIL S. ORENSTEIN (203), *Departments of Pathology, Beth Israel Hospital and Harvard Medical School, and the Charles A. Dana Research Institute, Beth Israel Hospital, Boston, Massachusetts 02215*

URSULA OTZ (1), *Institute of Clinical Immunology, Inselspital, Bern 3010, Switzerland*

DANIELE PRIMI (273), *Department of Microbiology and Immunology, University of California, San Francisco, California 94143*

HEINZ G. REMOLD (153), *Departments of Medicine, Harvard Medical School and the Robert B. Brigham Division of Brigham and Women's Hospital, Boston, Massachusetts 02115*

ROSS E. ROCKLIN (163), *Department of Medicine, Tufts University School of Medicine–New England Medical Center Hospital, Boston, Massachusetts 02111*

MICHAEL R. RUFF (235), *Department of Immunology and Medical Microbiology, University of Florida College of Medicine, Gainesville, Florida 32610*

S. B. SALVIN (295), *Department of Microbiology, School of Medicine, University of Pittsburgh, Pittsburgh, Pennsylvania 15261*

LAWRENCE B. SCHOOK† (1), *Institute of Clinical Immunology, Inselspital, Bern 3010, Switzerland*

MAX H. SCHREIER (31), *Basel Institute for Immunology, 4005 Basel, Switzerland*

KENDALL A. SMITH (21), *The Immunology Program, Dartmouth Medical School, The Norris Cotton Cancer Center, Hanover, New Hampshire 03755*

RICHARD P. TRIGLIA (273), *Department of Microbiology and Immunology, University of California, San Francisco, California 94143*

PETER VOGT (125), *Institute for Microbiology, University of Basel, Basel, Switzerland*

LARRY M. WAHL (179), *Laboratory of Microbiology and Immunology, National Institute of Dental Research, National Institutes of Health, Bethesda, Maryland 20205*

SHARON M. WAHL (179), *Laboratory of Microbiology and Immunology, National Institute of Dental Research, National Institutes of Health, Bethesda, Maryland 20205*

† Present address: Department of Microbiology and Immunology, University of Michigan Medical School, Ann Arbor, Michigan 48109.

apparent selectivity for neoplastic cells are exciting themes for future research, as is here pointed out.

In Chapter 12 Primi *et al.* present a major and exciting technical advance in lymphokine research—the design of a method for enumerating lymphokine-secreting cells. Making use of the increasing availability of anti-lymphokine antibodies, the authors are the first to offer us a long-awaited nonimmunoglobulin equivalent of the Jerne plaque assay. They rightly point out the practically unlimited potential of this and analogous techniques, especially in light of the methodological revolution initiated by hybridoma-derived monoclonal antibodies.

The closing chapter is devoted to a key issue in lymphokine studies, the significance of the presence of lymphokines in the tissues of animals and humans. Neta and Salvin discuss the experimental and clinical demonstration of lymphokines, mostly of the inflammatory type, in blood, lymph, serous cavities, and even in the aqueous humor of the eye.

This second volume of *Lymphokines* begins more fully than its predecessor to reflect the extraordinary diversity of lymphokine research and the impressive implications these concurrent developments portend for the immunological community. It is in this context that we hope it fulfills the expectations of our audience, while accurately projecting the intentions of the Editors. I warmly thank this volume's authors for their patient and effective cooperation and the staff of Academic Press for their sustained encouragement and support.

EDGAR PICK

Preface

Volume 2 of *Lymphokines*[1] is published at a time when research on immunoregulatory cell products is penetrating every aspect of immunology. A considerable portion of the papers now being published in the major immunological journals deal with lymphokines, monokines, and other cytokines; all recent textbooks of immunology devote entire sections to soluble mediators of cellular immunity.

This volume opens with a systematic screening for lymphokine activities detectable in the supernatants of continuous lymphoid and myeloid cell lines, as described in the chapter by Schook and colleagues. Nine distinct biologic activities were examined from 24 cell lines. The effectiveness of B-cell lines in producing MIF, interferon, and mitogenic and macrophage cytotoxicity factors clearly refutes the commonly accepted but thoroughly misleading idea that lymphokines are to be defined as soluble T-cell products.

In Chapter 2 Smith reviews our present knowledge of T-cell growth factor (TCGF) as well as the prospects for future lymphokine studies stemming from the discovery and rapidly expanding application of TCGF. The conceptual revolution, precipitated by the discovery of TCGF, has infiltrated many key areas of immunological research. The discovery of TCGF enables us to maintain selected T cells proliferating continuously, a matter of enormous significance.

In Chapter 3 Schreier analyzes the central role played by T- and B-cell growth factors in the primary antibody response *in vitro*. By using cloned helper T cells, the author proposes that specific antigenic stimulation of helper T cells generates nonantigen-specific growth factors acting upon B cells stimulated by the same or an unrelated antigen.

The immune response *in vitro* is also the subject of Chapter 4 by Hoffeld and colleagues. They propose that the suppressive activity of macrophages in this system is mediated by oxygen reduction products and derivatives of arachidonic acid oxidation.

Feldmann and Kontiainen (Chapter 5) provide us with an up-to-date

[1] The main title of this serial has been simplified from *Lymphokine Reports* to *Lymphokines*. Our intent is to emphasize the orientation of this publication as a wide-ranging survey of progress and the definition of new directions in lymphokine studies, rather than the mere publication of current research reports. It bears special emphasis that *lymphokine* is utilized here in its broadest connotation, as covering the entire spectrum of soluble immunoregulatory products of lymphocytes, macrophages, and other cells.

analysis of the role of antigen-specific T-cell factors in both help and suppression. They rightly emphasize the urgent need for more evidence in support of a role of such factors in the *in vivo* reality. They also propose a model for the structure of antigen-specific factors, the essence of which is the presence of a constant region, determining the functional property of the molecule (help or suppression), and a variable region which binds to antigen.

The role of soluble factors in macrophage–T-cell interaction is discussed in Chapter 6 by Erb *et al.* The monokine, known as genetically related macrophage factor (GRF), is released by macrophages incubated with antigen and is distinguished by its capacity to induce antigen-specific helper T cells and by its possession of Ia antigens. The authors point out that both the Ia and the immunogenic fragments present on GRF may act in a macrophage-bound form.

In Chapter 7, the as yet little explored domain of the action mechanism of lymphokines is examined by Liu and Remold in their treatment of membrane receptors for lymphokines. In Chapter 8 Rocklin discusses the mechanism of action of leukocyte migration inhibitory factor (LIF). Liu and Remold describe in detail their own studies on the receptor for MIF/macrophage activating factor (MAF), which stemmed from the important discovery that simple sugars can block lymphokine action.

Rocklin gives a personal account of his studies of the mode of action of LIF. The possibility that a leukocyte-derived polypeptide, released under the influence of LIF, is actually mediating the cell-immobilizing effect of the mediator is considered.

The biological universality of lymphokines is aptly affirmed in the chapter by Wahl and Wahl on the modulation of connective tissue metabolism by lymphokines and monokines. The lymphokine-mediated interaction between lymphocytes and macrophages, at one end, and fibroblasts, at the other, may offer an explanation for many hitherto unexplained aspects of granuloma formation and, possibly, for the pathogenesis of some fibrotic conditions of obscure etiology.

Further proof of the penetration of lymphokine studies into many other areas of medicine is offered in Chapter 10 by Dvorak and colleagues, who analyze cytokine-like mediators secreted by malignant cells. Such mediators provide the tumor with a suitable microenvironment that neutralizes host defense mechanisms.

In Chapter 11 Ruff and Gifford offer a detailed description of tumor necrosis factor (TNF), a mediator discovered 20 years ago but representing the culmination of a research effort which started more than 100 years ago. The elucidation of its mechanism of action and of its

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Production of Lymphokines *in Vivo*

RUTH NETA AND S. B. SALVIN

Lymphokine and Monokine Activities in Supernatants from Human Lymphoid and Myeloid Cell Lines[1]

LAWRENCE B. SCHOOK,*,[2] URSULA OTZ,* SANDOR LAZARY,* ALAIN L. DE WECK,* JUN MINOWADA,† RANKA ODAVIC,‡ EVA MARIE KNIEP,§ AND VICTOR EDY**

*Institute of Clinical Immunology and ‡Division of Hematology, Inselspital, Bern, Switzerland; †Roswell Park Memorial Institute, Buffalo, New York; §Max-Planck Institut für Immunbiologie, Freiburg, Federal Republic of Germany; and **Theodor Kocher Institut, Bern, Switzerland

I. Introduction

During the past decade, an increasing number of soluble products from stimulated lymphocytes and monocytes referred to as "lymphokines" and "monokines," respectively, have been described (Wolstencroft and Dumonde, 1970; de Weck *et al.*, 1980). Classical approaches in the production of human lymphokines (LK) and

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[2] Recipient of Postdoctoral Research Fellowships awarded by the National Cancer Institute, NIH (CA-0628-01) and the Swiss National Fund (88.679.-0.78). Present address: Department of Microbiology and Immunology, University of Michigan Medical Center, Ann Arbor, Michigan 48109.

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monokines (MK) have involved the stimulation of peripheral blood lymphoid and myeloid cells, either specifically by antigen or nonspecifically by mitogens (Rocklin *et al.*, 1972; Remold *et al.*, 1972). One of the problems in using such unfractionated cell populations in the production of human LK and MK is a "ping-pong" effect in which stimulated cells foster the indirect production of LK or MK from other cell types (Oppenheim *et al.*, 1979). According to our experience (unpublished observations), this phenomenon leads to large variations in the production and pattern of LK and MK activities in supernatants from various individuals. Using Con A, one finds many activities in a given supernatant of human peripheral blood lymphocyte (PBL) stimulated cells such as migration inhibitory factor (MIF), mitogenic factor (MF), lymphocyte activating factor (LAF), and colony-stimulating activity (CSA), and although produced with different kinetics, it is virtually impossible to use mitogen stimulation for production of a single activity (Schook *et al.*, 1978). Second, the amounts of these biologically active products released from stimulated cells are extremely low and their frequently similar chromatographic and electrophoretic behavior creates difficulties during separation procedures (de Weck *et al.*, 1976). Earlier reports (Granger *et al.*, 1970; Papageorgiou *et al.*, 1974; Yoshida *et al.*, 1976) have shown that LK activities were found in supernatants from continuous lymphoid cell lines, which may be a source(s) of large amounts of LK. In an attempt to produce supernatants containing more restricted LK and MK activities, we have systematically assayed supernatants from 22 human lymphoid cell lines (LCL) and 2 myeloid cell lines (MCL) for nine various factors.

II. Materials and Methods

A. Maintenance of Lymphoid and Myeloid Cell Lines and Production of Lymphokine- and Monokine-Containing Supernatants

Twenty-two continuous lymphoid cell lines and two myeloid cell lines maintained at Roswell Park Memorial Institute were used in this study. A description of the individual line characteristics is given in Table I.

Supernatants for determination of LK and MK production were prepared as follows (Fig. 1): Initial stationary starter cultures (0.5×10^6 viable cells/ml) of RPMI 1640 supplemented with 10% heat-inactivated fetal calf serum (FCS), penicillin (100 U/ml), and strep-