Lymphokines

A Forum for Immunoregulatory Cell Products

EDITED BY
EDGAR PICK

Lymphokines

A Forum for Immunoregulatory Cell Products

EDITED BY

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

ADVISORY EDITOR
MAURICE LANDY
P O Box 2423
La Jolla, California

VOLUME 11

1985

ACADEMIC PRESS, INC. *Harcourt Brace Jovanovich, Publishers*
Orlando San Diego New York Austin
London Montreal Sydney Tokyo Toronto

ACADEMIC PRESS, INC
Orlando, Florida 32887

United Kingdom Edition published by
ACADEMIC PRESS INC (LONDON) LTD
24–28 Oval Road, London NW1 7DX

ISSN 0197-596X

ISBN 0–12–432011–2

PRINTED IN THE UNITED STATES OF AMERICA

85 86 87 88 9 8 7 6 5 4 3 2 1

Contributors

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

DOLPH O. ADAMS (157), *Departments of Pathology and Microbiology-Immunology, Duke University Medical Center, Durham, North Carolina 27710*

GEOFFREY J. BLACKWELL (187), *The Wellcome Research Laboratories, Department of Prostaglandin Research, Beckenham, Kent BR3 3BS, England*

ANTONIO CELADA (87), *Department of Immunology, Research Institute of Scripps Clinic, La Jolla, California 92037*

KLAUS-MICHAEL DEBATIN (119), *Institute of Immunology and Genetics, German Cancer Research Center, D-6900 Heidelberg, Federal Republic of Germany*

JOHN E. ESTES (213), *Department of Pharmacology and Cancer Cell Biology Program, Lineberger Cancer Research Center, University of North Carolina, Chapel Hill, North Carolina 27514*

TOMAS GANZ (171), *Department of Medicine, UCLA-Center for the Health Sciences, Los Angeles, California 90024*

DIETHARD GEMSA[1] (119), *Institute of Molecular Pharmacology, Medical School of Hannover, German Cancer Research Center, D-6900 Heidelberg, Federal Republic of Germany*

G. YANCEY GILLESPIE (213), *Department of Surgery and Tumor Immunology Program, Lineberger Cancer Research Center, University of North Carolina, Chapel Hill, North Carolina 27514*

RONALD H. GOLDFARB (157), *Department of Immunology and Infectious Disease, Central Research Division, Pfizer, Inc., Groton, Connecticut 06340*

PATRICK W. GRAY (1), *Genentech, Inc., South San Francisco, California 94080*

[1] Present address: Department of Medical Immunology, Medical Center for Hygiene, University of Marburg, D-3550 Marburg, Federal Republic of Germany.

A. HSU (243), *Divisions of Nephrology and Hypertension, Department of Internal Medicine, University of Cincinnati College of Medicine, Cincinnati, Ohio 45267*

HOWARD M. JOHNSON (33), *Department of Comparative and Experimental Pathology, The University of Florida, Gainesville, Florida 32610*

WILLIAM J. JOHNSON[2] (157), *Department of Pathology, Duke University Medical Center, Durham, North Carolina 27710*

BURAK KOZAN (119), *Institute of Molecular Pharmacology, Medical School of Hannover, German Cancer Research Center, D-6900 Heidelberg, Federal Republic of Germany*

PETER H. KRAMMER (119), *Institute of Immunology and Genetics, German Cancer Research Center, D-6900 Heidelberg, Federal Republic of Germany*

CLAIRE KUBELKA[3] (119), *Institute of Immunology and Genetics, German Cancer Research Center, D-6900 Heidelberg, Federal Republic of Germany*

ROBERT I. LEHRER (171), *Department of Medicine, UCLA-Center for the Health Sciences, Los Angeles, California 90024, and Department of Medicine, VA Medical Center, West Los Angeles, California 90073*

E. P. MACCARTHY (243), *Divisions of Nephrology and Hypertension, Department of Internal Medicine, University of Cincinnati College of Medicine, Cincinnati, Ohio 45267*

B. S. OOI (243), *Divisions of Nephrology and Hypertension, Department of Internal Medicine, University of Cincinnati College of Medicine, Cincinnati, Ohio 45267*

Y. M. OOI (243), *Divisions of Nephrology and Hypertension, Department of Internal Medicine, University of Cincinnati College of Medicine, Cincinnati, Ohio 45267*

LUCA PARENTE (187), *The Wellcome Research Laboratories, Department of Prostaglandin Research, Beckenham, Kent BR3 3BS, England*

[2] Present address: Department of Immunology, Smith, Kline and French Laboratories, Philadelphia, Pennsylvania 19101.

[3] Present address: Institute of Immunology and Serology, University of Heidelberg, D-6900 Heidelberg, Federal Republic of Germany.

W. J. PLEDGER (213), *Department of Pharmacology and Cancer Cell Biology Program, Lineberger Cancer Research Center, University of North Carolina, Chapel Hill, North Carolina 27514*

ERNST RINDERKNECHT (1), *Genentech, Inc., South San Francisco, California 94080*

JOHN W. SCHRADER (47), *The Walter and Eliza Hall Institute of Medical Research, Royal Melbourne Hospital, Victoria 3050, Australia*

ROBERT D. SCHREIBER (87), *Department of Immunology, Research Institute of Scripps Clinic, La Jolla, California 92037*

MICHAEL E. SELSTED (171), *Department of Medicine, UCLA-Center for the Health Sciences, Los Angeles, California 90024*

COSTA G. SEVASTOPOULOS (1), *Genentech, Inc., South San Francisco, California 94080*

MICHAEL P. SHERMAN (171), *Department of Pediatrics, UCLA-Center for the Health Sciences, Los Angeles, California 90024*

VALERIE VAN DYKE-PHILLIPS (157), *Department of Immunology and Infectious Disease, Central Research Division, Pfizer, Inc., Groton, Connecticut 06340*

JAN VILČEK (1), *Department of Microbiology, New York University Medical Center, New York, New York 10016*

GRACE H. W. WONG[4] (47), *The Walter and Eliza Hall Institute of Medical Research, Royal Melbourne Hospital, Victoria 3050, Australia*

[4] Present address: Department of Molecular Biology, Genentech, Inc., South San Francisco, California 94080.

Preface

> Good Heavens! For more than forty years I have been speaking prose without knowing it.
> (MOLIÈRE, *Le Bourgeois Gentilhomme*)

This volume of *Lymphokines* is, in a manner, an update of Volume 3 of this serial publication which dealt with Lymphokines in Macrophage Activation. As a hallmark of changing times, the deliberately vague term "lymphokines" can now be replaced by "interferon γ (IFN-γ)," a product bearing the distinctive marks of the protein aristocracy: a cloned gene and a complete amino acid sequence. Originally a stepchild, reluctantly accepted by old-school immunologists into the lymphokine community, IFN-γ became the enfant terrible of the family, outstanding in the multiplicity of its actions and the variety of its target cells. The original claim concerning IFN-γ that was not substantially modified by the influx of newer experimental data is that it is produced by T cells only, albeit not only in the course of a typical immune response. The field most thoroughly revolutionized by the changing view of IFN-γ was macrophage physiology. Faced with the irrefutable evidence of the IFN-γ nature of the macrophage activating factor (MAF), immunologists felt, just as Molière's hero did, that they were working for years with a well-defined lymphokine without being aware of it.

In the opening chapter to this volume, Vilček *et al.* most appropriately provide a complete and thought-provoking introduction to the immunoregulatory properties of human and murine IFN-γ. They point out that one of the central unsolved mysteries of interferon research is the finding that immune and nonimmune interferons share so many activities in spite of separate genes, structural differences, and distinct target cell receptors. Citing De Maeyer, Vilček *et al.* summarize this situation as follows: "IFN-γ is an immunomodulatory lymphokine which also exerts antiviral activity, while type I interferons are antiviral proteins which also can act as immunomodulators." In the subsequent chapter, Johnson discusses a rarely approached aspect of IFN-γ—the cellular mechanism of its production. The author proposes a model in which IFN-γ production is regulated by the interaction between helper, suppressor, and lymphokine-producing T cells. The helper cell effect is mediated by interleukin 2 (IL-2) that induces a sequence of

intracellular events culminating in IFN synthesis. The intracellular reaction chain is composed of Ca^{2+} influx, phospholipase activation, arachidonate release, leukotriene synthesis, an increase in cellular cyclic GMP, and the activation of cyclic GMP-dependent protein kinase. Macrophages could be involved in this process by providing interleukin 1 (IL-1) to stimulate IL-2 production by helper cells and by secreting leukotrienes that can act directly on the IFN-producing cell. The modulation of a wide range of phenotypic markers on cells within and outside the immune system by IFN-γ is described in great detail by Wong and Schrader. They find that IFN-γ induces the expression of H-2 antigens on all cell types examined. Class II MHC antigens are induced in a more restricted range of cells that, nevertheless, includes, in addition to macrophages, mast, endothelial, thyroid, and pancreatic cells, keratinocytes, fibroblasts, and astrocytes. The intriguing question of the nonimmunological functions of Ia antigens and the apparent inability of IFN-α/β to induce class II MHC antigens are also discussed. Wong and Schrader cite the evidence for an *in vivo* role of IFN-γ-mediated modulation of MHC antigens in phenomena such as allograft rejection, infection, and autoimmunity and then list a number of physiological factors capable of opposing IFN-γ action (growth factors, glucocorticoids, and prostaglandins). Concentrating on the macrophage-directed effects of IFN-γ, Schreiber and Celada review the evidence showing that IFN-γ, in both mouse and man, represents the major MAF for tumor cytotoxicity. They are careful not to exclude the possibility that non-IFN-γ MAFs exist, but they make the pertinent remark that proof of their reality will have to be based on biochemical and not merely functional criteria. The accumulating information on cellular receptors for IFN-γ and the evidence supporting the existence of distinct domains in the IFN-γ molecule responsible for specific biological effects are also dealt with. A somewhat different and more conservative view is expressed by Gemsa *et al.* who discuss the macrophage-activating activities of mouse T cell clone products. The spectrum of macrophage activities examined by the authors is very wide and includes purely biochemical parameters (RNA and protein synthesis, glucosamine incorporation), indicators of oxidative and arachidonic acid metabolism, functional assays, parasite and tumor cell killing, and suppressor activity. Different T cell clones produce lymphokines that have different and sometimes opposing effects on macrophages. Gemsa *et al.* do not argue with the view that IFN-γ has potent macrophage-activating properties, but they offer a series of arguments supporting the existence of non-IFN-γ MAFs among which are the persistence of tumor cytostasis-inducing activity in material

treated at pH 2.0 and the existence of a cytotoxicity-inducing factor resistant to anti-IFN-γ and acting as a second signal (similar to LPS). Both Schreiber and Celada and Gemsa *et al.* are sympathetic to the hypothesis that separate molecular domains are responsible for MAF and antiviral effects and that molecules incorporating the first but lacking the second domain may exist and be regarded erroneously as totally different from IFN-γ. The next two chapters deal with the molecular mechanisms by which activated macrophages destroy tumor and microbial targets. Johnson *et al.* provide an overview of work originating mainly from their own laboratories on the cytolytic serine protease secreted by activated murine macrophages capable of destroying neoplastic but not normal target cells. The authors describe the biochemical properties of the 36- to 40-kDa protease, the signals required for its secretion, and the substances that suppress its liberation. Although the natural trigger for the release of the cytolytic protease is contact of the macrophage with tumor cells, LPS and stimulation of the scavenger receptor for modified lipoproteins can act as alternative signals. Lehrer *et al.* describe their finding of two cationic peptides in rabbit alveolar macrophages with potent bactericidal, fungicidal, and antiviral activity which also act as nonspecific opsonins. The two peptides are each 33 amino acids long and differ from one another at only one residue. They are probably localized in primary and secondary lysosomes, and their cellular concentration is remarkably high. A question that Lehrer *et al.* intend to investigate in the future is the mechanism of the microbicidal effect. This issue is made especially intriguing in the light of the finding that the cationic peptides show similarity to certain neurotoxins of snake venoms. Macrophages are major producers of arachidonic acid metabolites, and this subject has been dealt with repeatedly in previous volumes of this serial publication. Now, Blackwell and Parente discuss the endogenous inhibitor of phospholipase activity known as macrocortin. The material is produced by macrophages, neutrophils, thymus cells, and renal medullary cells in response to glucocorticoids. The steroid was shown to stimulate both the synthesis and extracellular release of macrocortin following a pattern characteristic for secretory proteins. Because inhibition of phospholipase activity will have as one of its consequences the inhibition of eicosanoid synthesis, the antiinflammatory potential of macrocortin was examined. The authors report that macrocortin administered *in vivo* was indeed capable of reducing carrageenan-induced inflammation suggesting a second messenger role for the protein in the antiinflammatory effects of glucocorticoids. The final two chapters deal with the cytoregulatory role of macrophage-derived factors. Gil-

lespie *et al.* review the quite extensive literature on the elaboration by macrophages of growth factors for mesenchymal cells, principally fibroblasts. These factors act similarly to platelet-derived and fibroblast growth factors. The authors describe the properties of one particular monokine, termed macrophage-derived competence factor, and show that it is distinct from IL-1, another mitogenic monokine. Interestingly, there is evidence for independent modulation of the production of these two factors in the course of macrophage activation and stimulation. The final chapter by Ooi *et al.* provides the missing link between the research laboratory and the patient's bed. The authors discuss the involvement of monokines in the pathogenesis of nephritis. They show that macrophage supernatants contain factors that can either stimulate or suppress mesangial cell proliferation in culture. It was found that the suppressive effect is mediated by the stimulation of endogenous prostaglandin synthesis in the target cells. Subsequent studies demonstrated that human monocyte supernatants also exert a stimulatory effect on endothelial cell proliferation. The authors conclude that monocytes–macrophages that are present in the glomeruli of patients with nephritis are major mediators of glomerular hypercellularity and subsequent impairment of function.

This volume illustrates emphatically the changing scene of lymphokine research. The early descriptive period that was so essential in bringing lymphokines into the forefront of biomedical progress is ending. The future belongs to molecular and cell biology, and intracellular events at both the synthetic and target cell poles of lymphokine function will keep us busy for many years to come.

I thank the authors for their cooperation in making this book a reality, Dr. Steven B. Mizel for sharing the editorial task, and the staff of Academic Press who assisted the editors with their customary dedication and competence.

EDGAR PICK

Contents

Molecular Characterization of Interferon γ as a Macrophage Activating Factor

ROBERT D. SCHREIBER AND ANTONIO CELADA

T Cell Clones Secrete Lymphokines That Activate Different Macrophage Functions

DIETHARD GEMSA, BURAK KOZAN, CLAIRE KUBELKA, KLAUS-MICHAEL DEBATIN, AND PETER H. KRAMMER

Secretion of a Novel Cytolytic Protease by Activated Murine Macrophages: Its Properties and Role in Tumor Cytolysis

WILLIAM J. JOHNSON, RONALD H. GOLDFARB, VALERIE VAN DYKE-PHILLIPS, AND DOLPH O. ADAMS

Multipotent Antimicrobial Peptides of Activated Macrophages

ROBERT I. LEHRER, MICHAEL E. SELSTED, TOMAS GANZ, AND MICHAEL P. SHERMAN

Macrocortin, a Glucocorticoid-Induced Regulator of Cell Function

GEOFFREY J. BLACKWELL AND LUCA PARENTE

Macrophage-Derived Growth Factors for Mesenchymal Cells

G. YANCEY GILLESPIE, JOHN E. ESTES, AND W. J. PLEDGER

Cellular Interactions in Nephritis: Role of Mononuclear Cell Products

B. S. Ooi, E. P. MacCarthy, A. Hsu, and Y. M. Ooi

Interferon γ: A Lymphokine for All Seasons

JAN VILČEK,* PATRICK W. GRAY,† ERNST RINDERKNECHT,† AND COSTA G. SEVASTOPOULOS†

*Department of Microbiology, New York University Medical Center, New York, New York, and †Genentech, Inc., South San Francisco, California

I. Introduction

The molecule now referred to as interferon γ (IFN-γ) was first described by Wheelock (1965) as an IFN-like protein produced by human white blood cells exposed to the T cell mitogen, phytohemagglutinin (PHA). The original demonstration of the ability of PHA to induce interferon (IFN) was the result of a serendipitous observation. Wheelock's real aim had been to study IFN induction by viruses in white blood cells, and PHA was employed merely to agglutinate and remove red blood cells from the freshly collected human blood. However, cultures of human blood cells exposed to PHA in the absence of virus also produced a factor with antiviral activity. This factor was originally termed "IFN-like" by Wheelock because, unlike typical IFN, its activity was destroyed by exposure to pH 2.

Subsequently, other investigators confirmed that various mitogenic plant lectins and bacterial products stimulate IFN production in lymphocytes (Friedman and Cooper, 1967). It was also shown that IFN could be induced in sensitized lymphocytes exposed to specific antigens (Green *et al.*, 1969). However, very little progress had been made in the elucidation of the properties of the IFN derived from lymphocytes by mitogenic stimulation until Youngner and Salvin (1973) pointed out that, in addition to a lack of stability at pH 2, this IFN was antigenically distinct and possessed other unique properties. They proposed the term "type II IFN," in contradistinction to "type I IFN" typically produced by both

lymphoid or nonlymphoid cells in response to viruses or double-stranded RNA. [It is now known that type I IFNs are heterogeneous and include the large family of interferon α (IFN-α) proteins and at least one interferon β (IFN-β) protein.] The same authors also had pointed out that "type II IFN" is usually produced together with other lymphokine activities (Salvin *et al.*, 1973). Hence, the concept of IFN-γ as a member of the lymphokine family was established. At about the same time, Falcoff (1972) proposed the term "immune IFN," based on the unique induction mechanism and distinct physicochemical properties of IFN produced by lymphocytes after mitogenic stimulation.

Some years later, studies performed with crude or partially purified preparations of IFN-γ suggested that this IFN was more potent than IFN-α or IFN-β as an immunomodulatory agent (Sonnenfeld *et al.*, 1978) or as an inhibitor of tumor cell growth (Crane *et al.*, 1978; Blalock *et al.*, 1980; Rubin and Gupta, 1980). Recent studies with highly purified preparations have shown that the potency of IFN-γ as an immunomodulatory agent and the range of its activities on various immune functions exceed all earlier expectations. On the other hand, the potent direct inhibitory action on tumor cell growth and viability has not always been seen with pure IFN-γ.

It is evident that many different types of immunomodulatory activities observed in the past with various lymphokine preparations can now be ascribed to IFN-γ. One example is a lymphokine activity termed "macrophage activating factor" or MAF. For a long time, MAF activity was thought to be associated with a unique protein. However, most earlier reports of MAF activity can now be ascribed to IFN-γ (see below). Similarly, a T cell-derived lymphokine activity operationally termed "macrophage Ia$^+$-recruiting factor" most likely is one and the same as IFN-γ (see below). Another lymphokine activity described some time ago as "Fc receptor-augmenting factor (FRAF)" now also is known to be mediated by IFN-γ (see below). Although IFN-γ was originally embraced by virologists, its many demonstrated immunomodulatory activities have turned this lymphokine into an object of keen interest to immunologists.

Our review will concentrate on two areas in which startling progress has occurred within the last year or two, i.e., characteristics of the IFN-γ molecule and elucidation of its various immunoregulatory activities. Other aspects, including the cellular origin of IFN-γ, studies on IFN-γ receptors, and the possible role of IFN-γ in immune disorders, have been recently reviewed elsewhere (Kirchner and Marcucci, 1984; Epstein, 1984).