INTERNATIONAL UNION AGAINST CANCER
AUSTRALIAN CANCER SOCIETY

THE NATURE OF LEUKAEMIA

Proceedings of the International Cancer Conference

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Papers presented by invitation at the International Cancer Conference held in Sydney between March 13th and 17th, 1972 under the auspices of the International Union Against Cancer and the Australian Cancer Society.

EDITED BY P. C. VINCENT

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editor's preface

This volume collects together papers on the subject of the nature of leukaemia, invited for presentation at the International Cancer Conference in Sydney, Australia, in March, 1972. This conference, which was sponsored by the Australian Cancer Society and the International Union Against Cancer, had as its other theme melanoma and skin cancer, and invited papers on this subject are being published in a companion volume.

Early in planning for the conference the organizers decided on a choice of integrated symposia which would focus attention on those lines of research currently most likely to advance our understanding of the pathobiology of leukaemia. Among these were viral, immunological and kinetic studies, the growth of leukaemia cells in solid state tissue cultures and, in the clinical field, the nature and significance of leukaemic remissions and the behaviour of the disease in response to treatment.

This book is being published in time to be available at the conference, and I appreciate the effort made by authors whose papers are included to make their manuscripts available in time. Each author has reviewed his subject and added new and often unpublished data, thus giving a comprehensive but succinct view of his particular field.

I am also indebted to the N.S.W. Government Printer, Mr V. C. N. Blight, and his staff for their unfailing co-operation in the production of this book, and to the Scientific Sub-Committee of the Australian Cancer Society and the conference secretariat for their considerable help.

P. C. VINCENT, B.Sc.(med.), M.B., B.S., F.R.A.C.P.

Medical Research Department
Kanematsu Memorial Institute
Sydney Hospital

Editor

March, 1972

Contributors to this Volume

J. L. Amiel, M.D.,
Institut Gustave-Roussy et Institut de Cancérologie et d'Immunogénétique,
94 Villejuif, France.

A. G. Baikie, M.B., Ch.B. (Glas.), F.R.C.P.E., F.R.A.C.P., M.R.C.Path., M.R.C.P.A.,
Professor of Medicine,
University of Tasmania Department of Medicine,
Royal Hobart Hospital, Hobart, Tasmania, 7000.

T. R. Bradley, M.Sc. (Melb.), Ph.D. (Reading),
Department of Physiology, University of Melbourne,
Parkville, Victoria, 3052.

S. H. Chan, M.B., B.S.,
Postgraduate Scholar,
The Walter and Eliza Hall Institute of Medical Research,
Royal Melbourne Hospital,
Melbourne, Victoria, 3050.

Maria I. Colnaghi, Ph.D.,
Associate, Division Experimental Oncology A,
Istituto Nazionale per lo Studio e la Cura dei Tumori,
20133 Milano, Italy.

N. E. Day, Ph.D.,
Units of Biological Carcinogenesis and Epidemiology,
International Agency for Research on Cancer,
16, avenue Maréchal Foch,
69 Lyon 6e, France.

G. Della Porta, M.D.,
Director, Division Experimental Oncology A,
Istituto Nazionale per lo Studio e la Cura dei Tumori,
20133 Milano, Italy.

G. De Thé, M.D., Ph.D.,
Units of Biological Carcinogenesis and Epidemiology,
International Agency for Research on Cancer,
16, avenue Maréchal Foch,
69 Lyon 6e, France.

Maureen A. Entringer, B.S.M.T. (ASCP),
Research Technologist,
Section of Oncology, Division of Hematology,
University of Colarado Medical Center,
Denver, Colorado, 80220, U.S.A.

T. M. Fliedner, M.D.,
Professor,
Department of Clinical Physiology, Center of Basic Clinical Research,
University of Ulm,
Ulm (Donau), Germany.

Phyllis A. Fry, B.Sc. (Syd.), M.Sc. (W.A.),
Department of Physiology, University of Melbourne,
Parkville, Victoria, 3052.

A. Geser, M.D., M.Ph.,
Units of Biological Carcinogenesis and Epidemiology,
International Agency for Research on Cancer,
16, avenue Maréchal Foch,
69 Lyon 6e, France.

Oliver J. Glidewell,
Statistician,
Acute Leukemia Group B, Rosewell Park Memorial Institute,
Buffalo, New York, 14203, U.S.A.

F. W. Gunz, M.D., Ph.D., F.R.C.P., F.R.A.C.P., M.R.C.P.A.,
Director, Medical Research Department,
Kanematsu Memorial Institute,
Sydney Hospital, Sydney, New South Wales, 2000.

E. B. Harriss,
Department of Clinical Physiology, Center of Basic Clinical Research,
University of Ulm,
Ulm (Donau), Germany.

D. Hoelzer,
Department of Clinical Physiology, Center of Basic Clinical Research,
University of Ulm,
Ulm (Donau), Germany.

James F. Holland, M.D.,
Chief of Medicine A,
Rosewell Park Memorial Institute,
Buffalo, New York, 14203, U.S.A.

Yasuo Ichikawa, M.D.,
Institute for Virus Research, Kyoto University,
Sakyo-Ku, Kyoto, Japan.

Henry S. Kaplan, M.D.,
Professor and Chairman,
Department of Radiology, Stanford University School of Medicine,
Stanford University Medical Center,
Stanford, Calif., 94305, U.S.A.

Sven-Aage Killmann, M.D., Ph.D.,
Head, Division of Hematology,
Department of Medicine A, Rigshospitalet,
University Hospital of Copenhagen,
DK-2100, Copenhagen Ø, Denmark.

G. Klein, M.D.,
Institute for Tumor Biology, Karolinska Institutet,
Stockholm, Sweden.

Hilary Koprowski, M.D.,
Director,
The Wistar Institute of Anatomy and Biology,
36th and Spruce Streets,
Philadelphia, Pennsylvania, 19104, U.S.A.

B. Kuske,
Department of Clinical Physiology,
Center of Basic Clinical Research,
University of Ulm,
Ulm (Donau), Germany.

Lloyd W. Law, Ph.D.,
Laboratory of Cell Biology, National Cancer Institute,
National Institutes of Health,
Bethesda, Md., 20014, U.S.A.

Alvin M. Mauer, M.D.,
Professor of Pediatrics, University of Cincinnati, Ohio, and Director, Division of Hematology, The Children's Hospital and The Children's Hospital Research Foundation,
Cincinnati, Ohio, U.S.A.

E. A. McCULLOCH, M.D., F.R.C.P.(C),
Institute of Medical Science, University of Toronto, and Ontario Cancer Institute,
Toronto, Ontario, Canada.

D. METCALF, B.Sc. (Med.), M.D., M.C.P.A., F.A.A.,
Head,
Cancer Research Unit,
The Walter and Eliza Hall Institute of Medical Research,
Royal Melbourne Hospital,
Melbourne, Victoria, 3050.

J. F. A. P. MILLER, B.Sc., M.B., B.S., Ph.D., D.Sc., F.R.S., F.A.A.,
Head,
Experimental Pathology Unit,
The Walter and Eliza Hall Institute of Medical Research,
Royal Melbourne Hospital,
Melbourne, Victoria, 3050.

E. A. MIRAND, Ph.D., D.Sc.,
Associate Institute Director and Professor,
Rosewell Park Memorial Institute,
New York State Department of Health,
Buffalo, New York, U.S.A.

M. A. S. MOORE, M.A., D.Phil.,
Senior Research Fellow, Cancer Research Unit,
The Walter and Eliza Hall Institute of Medical Research,
Royal Melbourne Hospital,
Melbourne, Victoria, 3050.

ALVIN L. OTSUKA,
Research Fellow,
Section of Oncology, Division of Hematology,
University of Colorado Medical Center,
Denver, Colorado, 80220, U.S.A.

J. H. POPE, M.Sc., Ph.D.,
Queensland Institute of Medical Research,
Brisbane, Queensland, 4006.

BEVERLEY M. REEDMAN, B.Sc.,
Queensland Institute of Medical Research,
Brisbane, Queensland, 4006.

WILLIAM A. ROBINSON, M.D., Ph.D.,
Associate Professor of Medicine,
Section of Oncology, Division of Hematology,
University of Colorado Medical Center,
Denver, Colorado, 80220, U.S.A.

BARRY T. ROUSE, B.V.Sc., Ph.D.,
Post doctoral research fellow,
Laboratory of Immunogenetics,
The Walter and Eliza Hall Institute of Medical Research,
Royal Melbourne Hospital,
Melbourne, Victoria, 3050.

H. J. SEIDEL,
Department of Clinical Physiology, Center of Basic Clinical Research,
University of Ulm, Ulm (Donau), Germany.

J. SPRENT, B.Sc., M.B., B.S.,
Experimental Pathology Unit,
The Walter and Eliza Hall Institute of Medical Research,
Royal Melbourne Hospital,
Melbourne, Victoria, 3050.

EVAN R. STANLEY, Ph.D.,
Cancer Research Unit,
The Walter and Eliza Hall Institute of Medical Research,
Royal Melbourne Hospital,
Melbourne, Victoria, 3050.

MARGARET A. SUMNER, B.Pharm. (Lond.), M.Sc. (Melb.),
Department of Physiology, University of Melbourne,
Parkville, Victoria, 3052.

J. E. TILL, Ph.D.,
Institute of Medical Science, University of Toronto, and Ontario Cancer Institute,
Toronto, Ontario, Canada.

GEORGE J. TODARO, M.D.,
Chief,
Viral Leukemia and Lymphoma Branch,
National Cancer Institute,
National Institutes of Health,
Bethesda, Md., 20014, U.S.A.

PAUL C. VINCENT, B.Sc.(med.), M.B., B.S., F.R.A.C.P.,
Clinical Haematologist, Medical Research Department,
Kanematsu Memorial Institute,
Sydney Hospital, Sydney, New South Wales, 2000.

MARILYN K. WALTERS, B.Sc.,
Queensland Institute of Medical Research,
Brisbane, Queensland, 4006.

NOEL L. WARNER, Ph.D.,
Senior Research Fellow,
Laboratory of Immunogenetics,
The Walter and Eliza Hall Institute of Medical Research,
Royal Melbourne Hospital,
Melbourne, Victoria, 3050.

N. WILLIAMS, B.Sc.,
Cancer Research Unit,
The Walter and Eliza Hall Institute of Medical Research,
Royal Melbourne Hospital,
Melbourne, Victoria, 3050.

Contents

Induction of Murine Leukaemia: Interaction of Viruses, Target Cells, Host Factors, and Exogenous Agents

HENRY S. KAPLAN

INTRODUCTION

Although the mechanisms of action of the RNA tumour viruses are now being pursued at the cellular and even the molecular level *in vitro*, the very elegance of the newer methods carries the risk that it may tempt us to oversimplification. Accordingly, in opening this session on the murine leukaemia viruses, it seemed desirable to present a panoramic view of the evolution of this field of investigation at the *in vivo* level, to emphasize the complexity of the natural system within which these tumours develop.

The term "leukaemia" has been used to refer to a variety of neoplasms of the lymphatic and haematopoietic systems of the mouse. The most intensively studied of these is a diffuse, poorly differentiated lymphocytic lymphosarcoma, which occurs in virtually all strains of mice. In most strains, it tends to arise in the thymus gland and to disseminate secondarily to other lymphatic tissues, to major viscera, and to the bone marrow, with the preterminal development of frank lymphatic leukaemia in those animals which survive sufficiently long. In some high-leukaemia strains, notably AKR and C58, these tumours arise "spontaneously". Most other strains exhibit a very low spontaneous incidence but are susceptible to the development of an equally high incidence after exposure to ionizing radiations or a variety of chemical agents.[1]

VIRAL ETIOLOGY OF THE MOUSE LEUKAEMIAS

1. "SPONTANEOUS" LEUKAEMIAS

Gross [2] first succeeded in demonstrating the presence of a viral agent in the spontaneous lymphomas of the high-leukaemia AKR and C58 strains. By inoculating cell-free extracts prepared from these tumours into newborn C_3H mice, he induced a high incidence of identical tumours in this normally low-leukaemia strain. The agent could be propagated from one tumour generation to the next by serial passage, with progressive increases in tumour incidence and reduction in latent period. In time, the agent was purified, identified as a type C virus by electron microscopy, and extensively characterized biophysically and immunologically.

2. RADIATION-INDUCED LYMPHOMAS

The demonstration by our group [3 4 5] that thymic grafts restore the incidence of lymphomas in thymectomized, irradiated C57BL mice and in their F_1 hybrids, that the tumours thus induced originate in the

unirradiated thymus grafts, and that most of the tumours can be shown to be of donor genotype provided conclusive proof of the existence of a completely indirect induction mechanism. This paradox led to a search, which soon proved successful,[6 7 8] for the presence of leukaemogenic viruses in extracts of radiation-induced lymphomas. The agent thus recovered was again concentrated by serial passage, purified, and demonstrated by electronmicroscopy to have the morphology of a type C virus,[9] essentially indistinguishable from that of the Gross virus, and was named the "Radiation Leukaemia Virus" (RadLV). It is now evident that this was a misnomer, since the leukaemia virus which has been extracted from chemically-induced lymphomas of strain C57BL[10] is almost certainly the same virus. This development, as well as the growing profusion of other leukaemia viruses named for their discoverers, some of which may be redundant, points up the need for a new system of nomencalture for the murine leukaemia viruses.

3. Chemically-induced Lymphomas

Leukaemogenic agents, again presumably viral in nature, have now been extracted from lymphomas induced by virtually all of the known chemical leukaemogens, including the hydrocarbons,[10 11 12] urethane,[11] and nitroquinoline oxide.[13] Moreover, the group-specific antigen characteristic of the murine leukaemia viruses has been detected in chemically-induced lymphomas.[14] Thus, viruses appear to be associated with all of the murine lymphomas and lymphatic leukaemias, whether "spontaneous" or induced by external physical or chemical agents.

THE MULTIPLE COMPONENTS OF THIS ONCOGENIC SYSTEM AND THEIR INTERACTIONS

There are at least five major components of this oncogenic system: (1) the virus; (2) the thymus; (3) the bone marrow; (4) constitutional and environmental factors affecting the host; and (5) external physical or chemical inducing agents. The complex interactions of these components have now been partially elucidated *in vivo*.[1]

1. The Radiation Leukaemia (RadLV)

Advantage has been taken of the discovery that the virus is most efficiently propagated by direct intrathymic inoculation[15] to study its intrathymic replication by electronmicroscopy[16] and to obtain consistently high titer preparations for both *in vivo* and *in vitro* studies. It is a spherical-type C virus, with a diameter of approximately 110 mμ and a relatively thick lamellated outer layer, a dense inner membrane, and a nucleoid of relatively low density.[16] It has been purified from mouse lymphoma tissue and from rat plasma on sucrose gradients, in which it has a buoyant density of approximately 1·16, identical to that of the other known murine leukaemia viruses. It appears to be a single-stranded RNA-containing virus, on the basis of its ribonuclease-sensitive red fluorescence with acridine orange and *in vitro* labelling with tritiated uridine, and its RNA has a sedimentation rate of 71S, like that of RSV (RAV_1), with an associated molecular weight of about $1{\cdot}0–1{\cdot}2 \times 10^7$ daltons (E. F. Walker and H. S. Kaplan, unpublished data).

Although the neo-antigens which it induces on lymphoid tumour cells are indistinguishable from those induced by the Gross virus,[17] the host

ranges (strain specificity patterns) of the two viruses are strikingly different.[1] "Wild-type" RadLV is active in strains C57BL, BALB/c, and their hybrids, and relatively inert in strains C_3H, CBA, and AKR; conversely, "wild-type" Gross virus is active in the latter strains and inert in the former.

Although RadLV, like the Gross virus, is vertically transmitted through the embryo in its strain of origin, C57BL, it is not extractable in active form from C57BL embryo or neonatal tissues. Yet virus particles of similar morphology have been observed by electronmicroscopy in the foetal C57BL thymus.[18] The virus ultimately emerges spontaneously in the tissues of extremely old C57BL mice,[19 20] but appears to require the action of external physical or chemical agents for its activation and release from the tissues of younger animals.[21 22 23]

The host range of RadLV appears to undergo modulation after serial passage, perhaps as a result of phenotypic mixing. After serial passage in C57BL, it becomes active in the AKR strain, from which it can again be recovered in a form which remains active in strain C57BL; conversely, after adaptation to and serial passage in the SJL/J strain, it acquires sustained activity in the latter strain but loses activity in its strain of origin, C57BL.[1]

Although RadLV is appreciably more difficult than most of the other murine viruses to propagate *in vitro*, it has been shown to have the capacity to rescue defective murine sarcoma virus (MSV) from hamster tumour cell cultures,[24] and this property has been made the basis of an *in vitro* assay for RadLV.[25 26] The pseudotype of MSV resulting from the helper action of RadLV appears to be specific, since it shares the host-range preference of RadLV for strains C57BL and BALB/c.

Prior inoculation of certain other closely related viruses into young C57BL mice can strikingly inhibit the leukaemogenic activity of RadLV inoculated some weeks later.[28] Such inhibitory action has been demonstrated with "wild-type" Gross/AKR virus, with SJL-adapted RadLV, and with the RadLV pseudotype of MSV. Heat inactivation of Gross virus abolishes its inhibitory activity, suggesting that replication of the virus is essential to the inhibitory response. That viral interference by competition for specific sites on the target cell membrane is not a likely explanation is indicated by the finding that incubation of thymus lymphoid target cells with Gross virus *in vitro*, prior to their incubation with RadLV, yields the same tumour incidence as cell preparations incubated with RadLV alone. The alternative hypothesis that the responsible mechanism is immunologic is supported by the observation that antisera from C57BL mice previously inoculated with MSV-(RadLV) exhibit virus-neutralizing activity against appropriate dilutions of RadLV. From the fact that earlier attempts to immunize mice against their own viruses have failed, it has been presumed that they are tolerant to these vertically transmitted agents. If so, then perhaps we have found a way to break tolerance by the inoculation of other, very closely related viruses.

2. The Thymus

The thymus is essential for the induction of lymphomas in most strains of mice. The development of these tumours is virtually abolished by thymectomy and restored by the implantation, either subcutaneously or intrarenally, of thymic grafts.[3 15] The thymus is also the locus of genetic susceptibility to the induction of lymphomas [5] and of the direct oncogenic

action of the virus.[15] There is now abundant evidence that the thymus provides at least two quite different components to this tumour-induction system: (1) the lymphoid target cells, which undergo neoplastic transformation as a consequence of the action of RadLV; and (2) the epithelial-reticular stromal environment, which is essential in most strains for the progression of preneoplastic target cells to an autonomous, fully neoplastic state.

(a) *The target cells.* The immature lymphoblastic cells of the outer cortex of the neonatal thymus are the most abundant known source of target cells.[28] The relative abundance and susceptibility of target cells in the thymus are strongly influenced by their state of differentiation, as well as by the age and genetic constitution of the host animal.[28 29 30] When parental strain thymus grafts are implanted intrarenally in thymectomized, irradiated F_1 hybrid host animals and the subsequent direct intrathymic injection of RadLV is delayed for different intervals of time, the genotype of the resulting tumours corresponds closely to the time course of the shift from donor- to host-type cells in such grafts,[1 31] suggesting that the virus must act very rapidly to alter the reproductive behaviour of the lymphoid cells that it infects at the time of injection.

(b) *The epithelial-reticular thymic stroma.* If the neoplastic transformation induced by RadLV were an abrupt, one-step process, it might have been expected that infected target cells would require nothing more than a histocompatible host to proliferate into frank neoplasms. Moreover, if this process is inhibited by immunologic surveillance mechanisms, it should have been stimulated by prior thymectomy of the host. Paradoxically, however, susceptible thymic target cells infected with active preparations of RadLV and injected intrasplenically into thymectomized, histocompatible hosts yield no lymphomas unless susceptibility is restored to the host animals by the implantation of thymic grafts. That the intrasplenically-inoculated target cells migrate to the intrarenal thymic grafts is strongly suggested by the observation that the thymic grafts, when re-excised and reimplanted into secondary, intact hosts 15 to 60 days later, give rise to a high incidence of lymphomas. In contrast, the animals from which the thymic grafts are removed, even as late as 60 days after initial injection of infected target cells, fail to develop lymphomas unless they receive a new thymus graft. It appears that the infected target cells must remain for a surprisingly long period of time in intimate proximity to the epithelial-reticular cells of the thymus in order to undergo progression to an autonomous, fully neoplastic state. The nature of this transition is as yet unknown, but it is well-established that thymuses of certain genetic constitution provide a significantly more favourable environment than others.[28]

3. The Bone Marrow

The bone marrow is a principal site for storage of the RadLV in its oncogenically inert state. Large numbers of virus particles have been noted by electronmicroscopy in cytoplasmic vesicles within megakaryocytes.[28]

Intact bone marrow appears to exert a strong inhibitory influence on the development of lymphomas in mice treated with X-irradiation [33] or with hydrocarbon carcinogens.[34] The anti-leukaemogenic action of the