Applied Virology

Editor
EDOUARD KURSTAK

WIDAD AL-NAKIB

CHRISTINE KURSTAK

Applied Virology

Editor

EDOUARD KURSTAK
Faculty of Medicine
University of Montreal
Montreal, Quebec, Canada

Associate Editors

WIDAD AL-NAKIB
Faculty of Medicine
Kuwait University
Safat, Kuwait

CHRISTINE KURSTAK
Hospital Hôtel-Dieu
University of Montreal
Montreal, Quebec, Canada

1984

ACADEMIC PRESS, INC.
(Harcourt Brace Jovanovich, Publishers)

Orlando San Diego New York London
Toronto Montreal Sydney Tokyo

ACADEMIC PRESS, INC.
Orlando, Florida 32887

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

Library of Congress Cataloging in Publication Data

Main entry under title:

Applied virology.

Includes index.
1. Viral vaccines. 2. Virus diseases. I. Kurstak, Edouard.
QR189.5.V5A66 1984 616.9'2505 84-9272
ISBN 0-12-429601-7 (alk. paper)

PRINTED IN THE UNITED STATES OF AMERICA

84 85 86 87 9 8 7 6 5 4 3 2 1

Contributors

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

J. Abb (191, 365), Max von Pettenkofer Institute for Hygiene and Medical Microbiology, University of Munich, 8000 Munich 2, Federal Republic of Germany

N. Ajjan (241), Mérieux Institute, 69223 Lyon, Cedex 1, France

C. Algan[1] (159), Regional Office for Africa, World Health Organization, Brazzaville, Congo (Popular Republic)

J. W. Almond (31), Department of Microbiology, University of Leicester, Leicester LE1 7RH, England

W. Al-Nakib (401), Department of Microbiology, Faculty of Medicine, Kuwait University, Safat, Kuwait

F. Assaad (131), Division of Communicable Diseases, World Health Organization, 1211 Geneva 27, Switzerland

Lorne A. Babiuk (349, 431), Department of Veterinary Microbiology, Western College of Veterinary Medicine, and Veterinary Infectious Disease Organization, University of Saskatchewan, Saskatoon, Saskatchewan S7N 0W0, Canada

J. E. Banatvala (221), Department of Virology, St. Thomas' Hospital and Medical School, London SE1 7EH, England

T. A. Bektimirov (131), Virus Diseases Unit, World Health Organization, 1211 Geneva 27, Switzerland

Jennifer M. Best (221), Department of Virology, St. Thomas' Hospital and Medical School, London SE1 7EH, England

R. Branche (241), Mérieux Institute, 69223 Lyon, Cedex 1, France

F. Brown (17), The Animal Virus Research Institute, Pirbright, Surrey GU24 0NQ, England

[1]Present address: WHO Program Coordinator, P.O. Box 362, Antananarivo, Madagascar.

A. J. Clayton (123), Laboratory Centre for Disease Control, Ottawa, Ontario K1A 0L2, Canada
F. Deinhardt (191, 365), Max von Pettenkofer Institute for Hygiene and Medical Microbiology, University of Munich, 8000 Munich 2, Federal Republic of Germany
D. M. A. Evans (31), Division of Viral Products, National Institute for Biological Standards and Control, Holly Hill, Hampstead, London NW3 6RB, England
Morag Ferguson (31), Divison of Viral Products, National Institute for Biological Standards and Control, Holly Hill, Hampstead, London NW3 6RB, England
A. Paul Fiddian (103), Department of Clinical Immunology and Chemotherapy, Clinical and Applied Research Division, Wellcome Research Laboratories, Beckenham, Kent BR3 3BS, England
T. H. Flewett (343), Regional Virus Laboratory, East Birmingham Hospital, Birmingham B9 5ST, England
P. Fournier (241), Mérieux Institute, 69223 Lyon, Cedex 1, France
G. J. Galasso[2] (87), Development and Applications Branch, Microbiology and Infectious Diseases Program, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland 20205
Henrik Garoff (51), European Molecular Biology Laboratory, 6900 Heidelberg, Federal Republic of Germany
V. Gauss-Müller (365), Max von Pettenkofer Institute for Hygiene and Medical Microbiology, University of Munich, 8000 Munich 2, Federal Republic of Germany
P. D. Griffiths (401), Virus Laboratory, Royal Free Hospital and School of Medicine, Hampstead, London NW3 2QG, England
Colin R. Howard (171, 327), Department of Medical Microbiology, London School of Hygiene and Tropical Medicine, London WC1E 7HT, England
W. Jilg (191), Max von Pettenkofer Institute for Hygiene and Medical Microbiology, University of Munich, 8000 Munich 2, Federal Republic of Germany
Leevi Kääriäinen (51), Recombinant DNA Laboratory, University of Helsinki, 00380 Helsinki, Finland
C. Kurstak (479), Laboratory of Virology, Hospital Hôtel-Dieu de Montreal, University of Montreal, Montreal, Quebec H2W 1T8, Canada
E. Kurstak (479), Comparative Virology Research Group, Department of

[2]Present address: Office of Extramural Research and Training, National Institutes of Health, Bethesda, Maryland 20205.

Microbiology and Immunology, Faculty of Medicine, University of Montreal, Montreal, Quebec H3C 3J7, Canada

Kenneth Lundström (51), Recombinant DNA Laboratory, University of Helsinki, and Research Laboratory of State Alcohol Company, 00100 Helsinki, Finland

Raymond G. Marusyk (211), Neurovirology Research Unit, Department of Medical Microbiology, Faculty of Medicine, University of Alberta, Edmonton, Alberta T6G 2H7, Canada

P. D. Minor (31), Division of Viral Products, National Institute for Biological Standards and Control, Holly Hill, Hampstead, London NW3 6RB, England

Thomas P. Monath (377), Division of Vector-Borne Viral Diseases, Center for Infectious Diseases, Centers for Disease Control, U.S. Public Health Service, Department of Health and Human Services, Fort Collins, Colorado 80522

B. Montagnon (241), Mérieux Institute, 69223 Lyon, Cedex 1, France

F. A. Murphy (17), Division of Viral Diseases, Center for Infectious Diseases, Centers for Disease Control, Atlanta, Georgia 30333

Bruce L. Nicholson (445), Department of Microbiology, University of Maine at Orono, Orono, Maine 04469

Erling Norrby (3), Department of Virology, School of Medicine, Karolinska Institute, SBL, S-10521 Stockholm, Sweden

Siobhan O'Shea (221), Department of Virology, St. Thomas' Hospital and Medical School, London SE1 7EH, England

J. F. Obijeski (17), Genentech, Inc., South San Francisco, California 94080

Nils Oker-Blom[3] (51), Department of Virology, University of Helsinki, 00290 Helsinki, Finland

Ilkka Palva (51), Recombinant DNA Laboratory, University of Helsinki, 00380 Helsinki, Finland

Thomas W. F. Pay (73), Foot-and-Mouth Disease Vaccine Division, Wellcome Biotechnology Ltd., Pirbright, Surrey GU24 0NQ, England

Ralf F. Pettersson (51), Recombinant DNA Laboratory, University of Helsinki, 00380 Helsinki, Finland

Alfred M. Prince (199), Laboratory of Virology, The Lindsley F. Kimball Research Institute of the New York Blood Center, New York, New York 10021

M. Roggendorf (191, 365), Max von Pettenkofer Institute for Hygiene and Medical Microbiology, University of Munich, 8000 Munich 2, Federal Republic of Germany

[3]Present address: Office of the Chancellor, University of Helsinki, 00170 Helsinki, Finland.

M. Roumiantzeff (241), Mérieux Institute, 69223 Lyon, Cedex 1, France
R. Scheid (365), Max von Pettenkofer Institute for Hygiene and Medical Microbiology, University of Munich, 8000 Munich 2, Federal Republic of Germany
G. C. Schild (31), Division of Viral Products, National Institute for Biological Standards and Control, Holly Hill, Hampstead, London NW3 6RB, England
Robert E. Shope (231), Department of Epidemiology and Public Health, Yale Arbovirus Research Unit, Yale University School of Medicine, New Haven, Connecticut 06510
M. Spitz (31), Immunology Section, National Institute for Biological Standards and Control, Holly Hill, Hampstead, London NW3 6RB, England
W. Stephan (63), Biotest Pharma GmbH, D-6000 Frankfurt am Main, Federal Republic of Germany
P. Tijssen (479), Comparative Virology Research Group, Department of Microbiology and Immunology, Faculty of Medicine, University of Montreal, Montreal, Quebec H3C 3J7, Canada
P. Trotemann (241), Mérieux Institute, 69223 Lyon, Cedex 1, France
G. van der Groen (299), Institute of Tropical Medicine, B-2000 Antwerp, Belgium
M. H. V. Van Regenmortel (463), Institute of Molecular and Cellular Biology, 67000 Strasbourg, France
J. C. Vincent-Falquet (241), Mérieux Institute, 69223 Lyon, Cedex 1, France
K. von der Helm (365), Max von Pettenkofer Institute for Hygiene and Medical Microbiology, University of Munich, 8000 Munich 2, Federal Republic of Germany
P. R. Young (327), Department of Medical Microbiology, London School of Hygiene and Tropical Medicine, London WC1E 7HT, England
R. Zachoval (191), Max von Pettenkofer Institute for Hygiene and Medical Microbiology, University of Munich, 8000 Munich 2, Federal Republic of Germany
G. Zoulek (191), Max von Pettenkofer Institute for Hygiene and Medical Microbiology, University of Munich, 8000 Munich 2, Federal Republic of Germany
Arie J. Zuckerman (171), Department of Medical Microbiology, London School of Hygiene and Tropical Medicine, London WC1E 7HT, England

Preface

The chief aim of "Applied Virology" is to discuss the practical applications of recent developments in basic virology, not only to virology but to other disciplines as well, and to demonstrate the impact of virus diseases on the environment, economy, and the health of man, animals, and plants. The chapters in this volume, written by well-known experts, provide a large body of practical information on the modern strategy used to produce virus vaccines, on antiviral chemical compounds, on simple, rapid, and specific diagnostic techniques, and on epidemiology in relation to the prevention and control of virus diseases. "Applied Virology" reflects the current tendency to produce safe and efficacious vaccines by genetic engineering technologies and considers their laboratory study and field applications. Noninfectious, synthetized peptides used as safe virus vaccines are reviewed with special attention to their immunogenicity, multispecificity, and usefulness in case of epidemics. A distinct but equally essential step for the study of epidemics and the control of diseases in relation to the variation among viruses is the rapid, sensitive, and reliable diagnosis of virus diseases. The applications of advanced technologies for this purpose are also discussed.

It is our hope that "Applied Virology" will be of use to all concerned with virus diseases, e.g., hospitals, veterinary clinics, infectious disease control centers, plant protection institutes, departments of agriculture and public health, and those who endeavor to improve the quality of life and environment by using advanced diagnostic and disease control technologies. It will be an aid to all virologists, and will fill the needs of those working in developing countries, in which virus diseases continue to be an unresolved health, economic, and environmental problem.

Virus diseases remain a worldwide problem, contributing to human disability and mortality and causing severe economic losses by affecting livestock and crops in all countries. Even with the preventive measures taken in the United States, losses due to viral diseases exceed billions of dollars annually. These costs are substantially higher in Africa, Asia, the Middle East,

and South and Central America, where virus diseases hamper the development of several countries.

I wish to express my sincere gratitude to the contributors for the effort and care with which they have prepared their chapters, to the Faculty of Medicine of University of Montreal and the Faculty of Medicine of Kuwait University for the help provided, and the staff of Academic Press for their part in the production of this volume.

Edouard Kurstak

Introduction

Biblical writings, ancient scripts, and archeological findings testify to the length of time virus diseases have scourged humanity. For example, examination of the 3000-year-old mummy Pharaoh Ramses V reveals pockmarks suggesting that he may have contracted smallpox, whereas Rhases, a Persian physician who lived about 1000 years ago, described how smallpox spread from Africa, where it was endemic, to Europe and the Middle East during the first millenium AD (Becker, 1983). A commemorative carving in stone, dating from the eighteenth Egyptian dynasty (1580–1350 BC), shows a young man with a withered and shortened leg typical of poliomyelitis virus paralysis (Melnick, 1984).

Virus diseases continue to contribute significantly to human morbidity or mortality and to cause severe economic loss by affecting livestock and crops, particularly in developing countries. For example, an estimated 200 million persons are chronically infected with hepatitis B virus, one million children are estimated to die annually from measles, five million persons are killed by acute gastroenteritis (mainly rotaviruses) annually, and the list goes on and on (Kurstak and Marusyk, 1984). The economic losses can be staggering (exceeding one trillion dollars in the United States alone). Those due to animal or plant viruses are equally enormous, and the diseases caused are a major factor in chronic malnutrition in many parts of the world. Reduction of crops by 75–90% by plant viruses occurs frequently, causing disruption of the fragile nutritional balance for large populations. Plant pathologists are at a disadvantage in protecting crops from virus infections since immunization is not possible. The main traditional strategies used to protect crops are cultivation measures such as starting with virus-free material, removal of vectors or diseased material, and restriction of epidemics through, e.g., crop rotation and plant or culture spacing. Chemotherapy has hitherto been impossible or impractical in this area, but new advances in this field may be most exciting in the area of plant virology.

Virology, as a science, has passed through a relatively long descriptive

phase, a shorter but explosive expansion phase over the last two decades in which advanced techniques in biochemistry, immunology, molecular biology, genetics, etc., provided deeper insights in the molecular structure, functioning, and reproduction of viruses, to a new phase: applied virology. Applied virology is a direct result of several breakthroughs in molecular biology, immunology, and diagnostic procedures. Wildy (1984) distinguished virologists, i.e., those who want to learn more about viruses ("second-phase virologists"), from "viropractors" ("third-phase virologists") whose purpose is to exploit viruses as tools. The latter are becoming very important in disparate areas such as genetic engineering, development of vaccines and diagnostic procedures, and insect control, and this group may, as Wildy (1984) pointed out, be growing faster than that of "pur sang" virologists.

Parallel with the development of virology, attempts have been made to combat virus diseases. A milestone in this respect was the large-scale production of poliovirus in cell cultures and its use, after formaldehyde inactivation, as a vaccine in the early 1950s by J. Salk. The subsequent discovery that the virus thus produced was sometimes contaminated with the undesirable SV40 virus (latent in cells), which can transform cells *in vitro,* necessitated the application of more rigorous control measures. Though inactivated viruses are efficient vaccines in cases in which viremia are an essential part of the disease, attenuated live viruses have been preferred to provide local immunity. Live vaccines have been established primarily by adaptation of the virus in question to an unnatural host. The attenuated virus may contain a large number of mutations, e.g., 35 for poliovirus type 1 vaccine (Nomato *et al.,* 1981). The attenuated virus may revert to the virulent strain again (e.g., 1 per 3×10^6 doses of live polio vaccine; CDC, 1982). Though in developed countries this vaccine has been widely used, it is less used in developing countries since it interferes with other enteric infections.

Attenuated live-virus vaccines are widely preferred, but they also carry significant risks. They may revert back to wild type or revert to novel strains with an unpredictable host range. Moreover a live-virus vaccine may be attenuated for one host but not for another, as exemplified with several attenuated live rabies vaccines. Emergence of novel strains is occurring at a higher rate than generally assumed and could potentially be very hazardous. Too often it is assumed that viruses have a certain constant host range and that each virus exhibits a distinct and unique antigenicity. Canine parvovirus for example demonstrates the fallacy of these "laws." In the Spring of 1978 articles appeared in both the lay and scientific press concerning the rapid spread of canine parvovirus, simultaneously in different regions, which caused a fulminant enteritis of high morbidity and mortality (up to 80%) in

dogs of all ages and myocarditis in young puppies. This virus not only caused a syndrome resembling the panleukopenia–enteritis syndrome in felines and mink (Kurstak and Tijssen, 1981), but the virus proved to be very closely related to the mink parvovirus (Tratschin *et al.*, 1982). This sudden host reversion of the virus from mink to dogs raises important questions with respect to the cause of this reversion, the possible reversion to man, and measures which can be taken to decrease the chances of such reversions. It should be stressed that the mink parvovirus is serologically undistinguishable from the feline panleukopenia parvovirus and that the mink parvovirus (mink enteritis virus) had an equally sudden outbreak in 1947 and spread from Canada rapidly around the world. The possibility that the canine parvovirus derives from the mink parvovirus must, therefore, be considered seriously. Adaptation could be a result of attempts to achieve attenuation of wild-type mink parvovirus. Retrospective analysis of sera revealed that the appearance of antibodies to this adapted virus in canine sera coincided with the disease, except in Belgium where some sera collected in 1976 and 1977 contained antibody (Schwers *et al.*, 1979). It is, however, difficult to understand how the virus could be present for two years without being detected since it is very pathogenic.

New horizons for the production of safe vaccines have been opened by new genetic engineering technologies. Genetic engineering techniques may be used to produce stable deletion mutants instead of point mutation strains which lose thereby a certain biological function but are still able to replicate in certain cells (Jones and Shenk, 1979). There is, nevertheless, a tendency to shift from whole-virus vaccines to vaccines of subunits or synthetic polypeptides. Despite an early view that the feasibility of the development of synthetic vaccines would be rather limited (Arnon, 1980), this approach has gained support in recent years. Current DNA technology also provides the tools to produce hybrid vaccines (e.g., recombinants of hepatitis B and vaccinia genes). Bacterial plasmids may be used as cloning vehicles for DNA and RNA viruses. Though these methods are still primarily used for the analysis of individual genes and the determination of their nucleotide sequences, cloning will have important applications with respect to the use of a single or a few viral gene(s) for the production of vaccines.

Another exciting avenue is the production of antiidiotype antibodies using hybridoma techniques. Monoclonal antibodies with an antiidiotype structure mimicking the crucial epitope on the virus seem to have great potential for immunization and protection of the individual from a virus without vaccination with the virus, one of its products, or synthetic epitopes of the virus (Kennedy *et al.*, 1984; Koprowski *et al.*, 1984).

In addition to the development of genetic engineering systems and

hybridoma techniques to develop vaccines and the synthesis of small peptides (epitopes) on a large scale, novel delivery systems and immunoenhancers are being developed.

These new technologies will not only have a tremendous impact on the knowledge, development, and quality of new viral reagents and vaccines but will also decrease very significantly the cost of the control of virus diseases. For example, it can be expected that vaccines which now cost more than $100, and therefore cannot be applied to control epidemics or endemics satisfactorily, can be produced for, e.g., 1% of the original cost, and are more effective than the original products. The worldwide excitement about these prospects hardly needs to be stressed.

It can also be seen that the comprehensive knowledge available on virus replication allows rational approaches to the design of antiviral drugs. Developments in this area will have important consequences, e.g., for the prophylaxis of viral diseases in plants. The number of antiviral drugs which have become available is still rather limited despite extensive efforts. The main problem is to find compounds which selectively inhibit the virus without having toxic effects on cells. Hitherto, research on antiviral drugs has been concentrated on a relatively few viruses (herpes, influenza), but it is expected that this number will increase rapidly due to recent advances in the understanding of virus replication and the development of sophisticated techniques. Though different stages of virus reproduction may be blocked, the stage of viral nucleic acid synthesis is most commonly chosen since the differences between viral enzymes and cellular enzymes may be exploited.

Breakthroughs in methodologies to diagnose virus diseases have been particularly prominent in the last few years and are expected to have a major impact on applied virology. The most important advances are due to the development of the hybridoma technique and the replacement of radiomarkers on antibodies or antigens by enzymes. Traditionally, radioimmunoassays have been used on a large scale, but important disadvantages hampered their transfer to less sophisticated laboratories or to developing countries. For example, expensive counters are needed and the radioactive reagents have generally short shelf lives. Moreover, the hazards of radioactive labels and the sophistication of the procedures and equipment restrict their use to well-equipped central laboratories in which large numbers of routine assays are performed. The enzyme immunoassays which found their inception in virology in 1969 (Kurstak *et al.*, 1969) are equally sensitive, are simple, and are performed with relatively very stable reagents. The costs are low so that these techniques are applicable anywhere. The rapid development of very specific reagents by the hybridoma technique will accelerate even more the already explosive expansion of this field.

The transfer of these new biotechnologies within developed countries and

to developing countries presents an enormous challenge and requires, in addition to suitable support, adjustments in education and infrastructure in order to prevent the establishment of heavy dependence on profit-oriented large companies or countries.

The new era of applied virology allows us to benefit from decades of fundamental and molecular research in biochemistry, immunology, and genetics of viruses. Without a doubt, applied virology has enormously increased the potential for the control of virus diseases. The establishment of adequate programs and the designation of necessary resources at national and international levels are required to achieve these goals.

Edouard Kurstak

REFERENCES

Arnon, R. (1980). *Annu. Rev. Microbiol.* **34**, 593–618.

Becker, Y. (1983). "Molecular Virology." Martinus Nijhoff, The Hague.

Centers for Disease Control (1982). *Morbidity and Mortality Weekly Report,* Jan. 29.

Jones, N., and Shenk, T. (1979). *Cell* **17**, 683–689.

Kennedy, R. C., Melnick, J. L., and Dreesman, G. R. (1984). *Science* **223**, 930–934.

Koprowski, H., Herlyn, D., Lubeck, M., DeFreitas, E., and Sears, H. F. (1984). *Proc. Natl. Acad. Sci. U.S.A.* **81**, 216–219.

Kurstak, E., Côté, J. R., and Belloncik, S. (1969). *C.R. Acad. Sci., Paris* **268**, 2309–2312.

Kurstak, E., and Tijssen, P. (1981). *In* "Comparative Diagnosis of Virus Diseases" (E. Kurstak and C. Kurstak, eds.), Vol. 3, pp. 3–65. Academic Press, New York.

Kurstak, E., and Marusyk, R. G. (1984). "Control of Virus Diseases." Dekker, New York.

Melnick, J. L. (1984). *In* "Karger Gazette," Issue 46–47, pp. 1–5.

Nomato, A., Kitamura, N., Lee, J. J., Rothberg, P. G., Imura, N., and Wimmer, E. (1981). *Virology* **112**, 217–227.

Schwers, A., Pastoret, P. P., Burtonboy, G., and Thiry, E. (1979). *Ann. Méd. Vét.* **123**, 561–570.

Tratschin, J. D., McMaster, G., Kronauer, G., and Siegl, G. (1982). *J. Gen. Virol.* **61**, 33–39.

Wildy, P. (1984). *In* "The Microbe 1984" (B. W. J. Mahy and J. R. Pattison, eds.), p. 1. Cambridge Univ. Press, Cambridge.

Contents