MEDICAL VIROLOGY III

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Editors

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Proceedings of the 1983 International Symposium on Medical Virology, held on October 19–21, 1983, in Anaheim, California, U.S.A.

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Elsevier
New York • Amsterdam • Oxford

Published by:

Elsevier Science Publishing Co., Inc.
52 Vanderbilt Avenue, New York, New York 10017

Sole distributors outside the USA and Canada:

Elsevier Science Publishers B.V.
P.O. Box 211, 1000 AE Amsterdam, The Netherlands

Library of Congress Cataloging in Publication Data

International Symposium on Medical Virology (1983: Anaheim, Calif.)
Medical virology III.

Includes indexes.
1. Virus diseases—Congresses. 2. Virology—Congresses. I. De la Maza, Luis M.
II. Peterson, Ellena M. III. Title. [DNLM: 1. Virology—congresses. W3
IN919JH 1983m / QW 160 1606 1983m]
RC114.167 1983 616.9'25 84-8188
ISBN 0-444-00829-2

Manufactured in the United States of America

FOREWORD

Interest in Medical Virology continues to increase at an accelerated rate. The aggressive therapeutic approach to some medical problems, e.g., organ failure, cancer, etc. has resulted in a large population of immunocompromised patients at a high risk of acquiring viral infections with high morbidity and mortality. In addition diseases of unknown origin, e.g., acquired immunodeficiency syndrome, where viruses are probably involved in their pathogenesis, have attracted the attention of the medical community and the general population.

Fortunately our increased knowledge of basic biochemical and genetic phenomena allows us to deal with these new challenges in a more rational manner than we did a while back. Highly effective diagnostic and therapeutic approaches are now available for viral infections. Tools that a few years ago were developed in the basic research laboratory are now in the hands of the practitioners of the medical sciences.

In this Symposium some of the new challenges and approaches to understand and treat viral infections are discussed. We hope our readers will find in this book a stimulus to pursue their goals in Medical Virology.

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Irvine, California, February, 1984

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ACKNOWLEDGEMENTS

We would like to thank all the participating speakers and Drs. Thomas C. Cesario and Jeremiah G. Tilles who moderated the sessions. Thanks also to Marie Pezzlo and Sandra Aarnaes who enthusiastically worked all year long preparing this meeting. Julie Gretz and Lisa Flynn provided the administrative and secretarial skills to bring this project to a happy end. Our appreciation to them too. Our deepest gratitude to the staff of the Medical Microbiology Division at the University of California Irvine for their continuous support and to Dr. Edward R. Arquilla for his guidance.

We are particularly grateful to the following organizations that through their financial back up made this meeting a reality:

Abbott Laboratories, Diagnostic Division
Bartels Immunodiagnostic Supplies, Inc.
California Laboratory Industries (Cal Labs)
Difco Laboratories
Dynatech Laboratories, Inc.
Electro-Nucleonics, Inc.
Flow Laboratories
General Diagnostics, Division of Warner-Lambert Co.
Hoechst-Roussel Pharmaceuticals, Inc.
Hoffman-LaRoche, Inc.
International Diagnostic Technology, Inc. (IDT)
M.A. Bioproducts
Marion Laboratories
Merck Sharp & Dohme, Postgraduate Program
Monsanto Co.
New England Nuclear
Ortho Diagnostic Systems, Inc.
Syva Co.

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INTERPRETIVE ASPECTS OF DIAGNOSTIC VIROLOGY

C. GEORGE RAY

INTRODUCTION

Over the past two decades, an amazingly rapid evolution of technology has brought medical virology to an important practical role in the diagnosis of human infections.

There have been many surprises, including the discovery of numerous "new" viruses; however, the issues go far beyond this. Among these are the increasing recognition of an immense diversity of clinical syndromes which can be caused by both "old" and "newer" viruses, and the ability of some to cause persistent infections, severe immunologic aberrations, or even malignancies. The difficulties are further compounded by the recent observations that different viruses may act together in a synergistic fashion to produce unique and sometimes severe disease in the host. A classical example is the recent discovery of delta virus and its interaction with hepatitis B virus.

We are now well into an era in which diagnosis is not only important, but where specific management or prevention of different viral infections is becoming more of a reality. Such therapy can be by means of chemicals (antivirals), specific immunologic manipulation, or other modalities. The important point is that it is reasonably certain that the control methods will vary according to the virus (or even the host), and that one needs to be certain what role a virus may have if detected in a patient.

Thus, the question has become more urgent - not only must we know how to detect viral infections, but we must be careful to properly interpret the meaning of a positive (or sometimes negative) result. Sometimes, this is not too difficult; for example, if a well described virus is isolated from an internal site such as a lung biopsy from a patient with disease at that site, and no other etiologic agent is also found, it seems reasonable to assume the virus is causative. However, the probabilities of association are better reinforced if we clearly understand the epidemiologic and biologic behavior of the virus in this situation, and are also able to demonstrate a host response to the infection.

In the remainder of this discussion, I will address possible ways to examine virologic results, and interpret their meaning in general as well as some specific terms.

Medical Virology III, de la Maza and Peterson, editors.

ESTABLISHING A RELATIONSHIP BETWEEN A VIRUS AND A CLINICAL DISEASE

The original postulates, as outlined by Jakob Henle and Robert Koch (Table I) were an excellent starting point in establishing associations between agents and disease states. However, if rigidly applied to modern virology, many of our current concepts of disease association would have to be discarded. It is essentially a fundamentalist approach, and I am reasonably certain that, if either Henle or Koch were here today, they would agree.

TABLE I

HENLE-KOCH POSTULATES[a]

1. The parasite occurs in every case of the disease in question and under circumstances which can account for the pathological changes and clinical course of the disease.

2. It occurs in no other disease as a fortuitous and nonpathogenic parasite.

3. After being fully isolated from the body and repeatedly grown in pure culture, it can induce the disease anew.

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[a] Based on Rivers' translation (1937).

Dr. Alfred Evans (1976) carefully considered what has occurred to change our thoughts about causation, and proposed a modern set of criteria for causation which I have only slightly paraphrased and listed as Evans' postulates in Table II. One might argue that even these criteria would be extremely difficult to totally fulfill in many cases. We must keep in mind that such fulfillment rarely occurs suddenly, but evolves. It often begins by describing a single event in which an agent was somehow associated, followed by further specific observations to see if a similar phenomenon recurs. If such appears to be the case, then the impetus for further, in-depth investigation is established, primarily utilizing prospective methods and open minds as to the validity of such observations. Controversy often occurs during this latter process; nevertheless, it is useful in provoking thoughtful planning of investigations and critical appraisal of our own results.

TABLE II

EVANS' POSTULATES: CRITERIA FOR CAUSATION

1. Prevalence of disease is higher in those exposed than in controls not exposed.

2. Exposure to the putative cause is more common in those with disease than in controls.

3. Incidence of disease is higher in those exposed to the putative cause than in those not exposed (prospective studies necessary).

4. Temporally, disease should follow exposure to putative cause with a distribution of incubation periods on a bell-shaped curve.

5. A spectrum of host responses should follow exposure to a putative agent along a logical biologic gradient from mild to severe.

6. A measurable host response following exposure to the putative cause should regularly appear in those lacking this before exposure, or should increase in magnitude if present before exposure.

7. Experimental reproduction of the disease should occur in higher incidence in animals or man appropriately exposed to the putative cause than those not exposed (may be deliberate or demonstrated in a controlled regulation of natural exposure).

8. Elimination of modification of the putative cause or its vector should decrease the incidence of the disease.

9. Prevention or modification of the host's response on exposure to the putative cause should decrease or eliminate the disease (e.g., immunization).

10. The whole thing should make biologic and epidemiologic sense.

From: Evans, 1976.

APPLICATION OF PROBABILITIES

Once the aforementioned criteria have been at least reasonably fulfilled, then it becomes possible to more precisely ascertain what the detection of a virus infection (either by culture, immunologic or biochemical methods) means to an individual patient. As a prelude to this, there are several general facts which need to be known.

1. Epidemiologic knowledge of the behavior of the virus in question. It is particularly important to have some ascertainment of how frequently one might expect to find asymptomatic infection in the population matched for

age, locale, time of year, socioeconomic status, and perhaps even sex. Thus, we have a risk of isolating a virus which has no role in the patient's illness (a "false positive").

2. Sufficient a priori evidence of the association of certain viruses with a specific clinical syndrome to allow a preliminary probability guess regarding the likelihood of detecting one of them. A simple illustration would be the detection of a coxsackie or echovirus in a throat swab from a child with aseptic meningitis, wherein the preliminary prediction that this would occur might have been 50%; conversely, isolation of herpes simplex or an adenovirus from the throat of this same patient would have been predicted to occur with a probability close to that seen in the "false positive" category, perhaps 5%. I will refer to this below as prevalence of the virus in the disease under investigation, or the best clinical guess of probability that the patient will have a specific virus (or one of several) at the site(s) sampled before the test is done.

3. Knowledge of the virus behavior in the host. These data provide additional information which serve to support or temper the conclusions in difficult interpretive situations. Such items include shedding from different sites, how long this may normally occur, host factors which may lead to virus reactivation, and the development of specific immune responses.

In the 18th century, the Reverend Thomas Bayes (1763) developed a probability theorem to attempt to solve problems such as those we are confronted with today. His work was published posthumously and has been much debated, used, and probably abused, since. In attempting to find a way to apply some statistical sense to the present subject, I turned to a simplified Bayesian model suggested by Katz (1974), and present it, with appropriate caveats. The model in this setting assumes two items which may not always be as valid as we would like: that the sensitivity of our detection system is high, and that the clinical and epidemiologic basis for guessing probabilities for a specific positive test result beforehand (prevalence of the virus) are sound. Assuming that we are reasonably close on these points, and also that we have some knowledge of the prevalence of false positives which might be expected in an individual patient, the following formula can be applied:

$$\text{Probability that disease is associated with virus detected} = \frac{1}{1 + \text{frequency of false positives} \times \left(\frac{1}{\text{prevalence of virus}} - 1 \right)}$$

Table III illustrates the various results one might expect from such an analysis.

TABLE III

PROBABILITY (AS PERCENTAGE) OF VIRUS ASSOCIATION WITH AN ILLNESS

Estimated Prevalence of Virus[a]	Frequency of False Positives	Probability of Association if Virus Detected
10	1	11
	10	10
	30	9
	50	7
25	1	33
	10	30
	30	26
	50	22
50	1	99
	10	91
	30	77
	50	67

[a] Preliminary probability guess that a specific virus (or one of several) will be detected (see text).

Using such an analysis enables us to appreciate the need for firm epidemiologic associations between specific viruses and the illnesses they may cause. Perhaps surprisingly to some, if that association is low to begin with (e.g., 10% in the left-hand column in Table III), it makes little difference whether the frequency of false positives is high or low - the probability of associating the virus with the patient's illness is poor, and can only be enhanced by other factors, such as isolation from a critical site (e.g., a lesion), excluding all other possible causes, and demonstrating temporally appropriate host immunologic response.

SEROLOGIC ASPECTS OF DIAGNOSIS

There are still situations where specific antibody determinations are useful or even necessary for both infection detection and interpretation of significance. These are summarized in Table IV.

TABLE IV

REASONS FOR SEROLOGICAL TESTING

	Situation	Examples
1.	Virus suspected, but not detected	Depends on clinical syndrome
2.	Virus detected, but etiologic significance is equivocal	Serological response to specific virus in question
3.	Virus suspected, but detection is difficult, unlikely, or slow	
	Myocarditis-pericarditis	Group B coxsackieviruses
	Hepatitis A	Hepatitis A virus
	Rubella	Rubella virus
	Central Nervous System Infections	Togaviruses Bunyaviruses Arenaviruses Measles Epstein-Barr virus
	Mononucleosis syndromes	Cytomegalovirus Epstein-Barr virus
4.	Immune status determination (single serum)	Rubella Hepatitis B Varicella-zoster

Assuming an appropriate level of test sensitivity and specificity, the most compelling serologic result is that in which there is a conversion from seronegativity (undetectable) to seropositivity in paired acute (or pre-illness) and convalescent sera. Alternatively, a fourfold or greater change in antibody titer is considered to be of significance; however, this may not always be so. For example, one can occasionally see "significant" rises in antibody titers to ubiquitous agents such as cytomegalovirus. These may be a result of stress reactivation by other agents, or perhaps by repeated subclinical exogenous reinfection (Waner et al. 1973).

In some cases, a single serum or serial samples may be examined for class-specific antibody titers (particularly IgM) and be well interpreted. Virus-specific IgM antibody usually rises early in the course of a primary infection and often persists for as long as four to six months before falling to undetectable levels. This has been used with notable success in the diagnosis of hepatitis A virus infections (Snydman et al. 1981), and to some extent for congenital or primary acquired cytomegalovirus infections in preg-

nancy (Griffiths et al. 1982a, 1982b). There are other infections where similar technology has been applied, including varicella-zoster, Epstein-Barr, rubella, and coxsackieviruses (Chernesky et al. 1982).

The IgM-specific testing has varied technical and interpretive limitations, depending upon the virus sought and the host involved. It is recognized that reactivation or reinfection may result in homotypic and even occasionally heterotypic IgM responses, particularly where herpesvirus group agents are involved (Chernesky et al. 1982; Sutton, 1979). In addition, some patients continue to produce IgM-specific antibody for more than six months after a primary infection with agents such as cytomegalovirus or rubella (Chernesky et al. 1982).

Single convalescent serum testing can sometimes be used to attempt a presumptive diagnosis. This requires adequate knowledge of the specific levels of antibody titers achieved in individuals during and after virologically proven acute infections, and should also be weighed against the knowledge of antibody titers in a well-matched, non-ill population. This approach has been used in outbreaks of enterovirus (Ray et al. 1966) and arthropod-borne virus infections (Sciple et al. 1968). For the most part, however, single-serum total antibody titer levels in individual patients should be interpreted with extreme caution.

False-negative serological results may also occur in some situations where significant infection is present. This may be related to the test system used, but can also be directly related to impaired host responses. Examples of the latter include some infections of the fetus or very young infant and severely immunocompromised patients.

In certain instances, the full interpretation of serological results may require testing for the presence of antibodies to several different structural or non-structural antigenic components of a virus. Two outstanding current examples of this are hepatitis B virus and Epstein-Barr virus. The complexities of testing and interpretation of hepatitis B testing have been detailed by Mushawar et al. (1981).

Henle and Henle (1981) have reviewed the diagnostic features and pitfalls of Epstein-Barr virus serologies, pointing out that the diagnosis is still mainly based on a combination of different antibody determinations. These are briefly summarized in Table V, and each can be described as follows: (1) IgG antibody to virus capsid antigen (VCA) appears early in infection and usually persists for life; (2) antibody to nuclear antigen appears 2 to 4 weeks after onset of primary infection and usually persists for life; (3) IgM antibody to VCA appears early in acute infection and usually persists for

only 2 to 4 months (however, reactivation of infection may provoke a similar response); (4) antibody to early antigen (EA) appears in most patients with infection and persists during the active phases of virus replication and symptoms (usually weeks to months). Anti-EA is usually directed at the diffuse (D) component of EA, but occasionally may be of the restricted (R) type. This latter response is more often described in patients with unusual or protracted courses of disease. Some patients have also been observed to lose detectable anti-EBNA in the course of a prolonged illness; the reasons for this are not known. As we learn more about Epstein-Barr virus infections, their ubiquity, and potential for bizarre manifestations, it becomes apparent that the serologic tests, while helpful, may not fully explain causation of the disease in question. Until more data are available, we have adopted a cautious attitude in those unusual disease conditions, in that we use the serologic battery to support a possible association, but also make reasonable efforts to exclude other possibilities. This often includes long-term clinical follow-up and serial serologic testing (Ray et al. 1982).

TABLE V

EPSTEIN-BARR VIRUS SEROLOGIC INTERPRETATIONS

Diagnostic Category	Antibodies Present (+) or Absent (-)			
	IgG-VCA	EBNA	EA	IgM-VCA
No past infection	-	-	-	-
Acute infection	+	-	+(90%)	+
Convalescent phase	+	+	+ or -	+ or -
Past infection	+	+	-	-
? Chronic or reactivation	+	+	+	-

Another serologic maneuver which deserves some comment is the simultaneous comparison of serum antibody titer levels with antibody levels in fluids from clinically affected body compartments, such as the central nervous system. The hypothesis is that local antibody production will result in higher levels than what might be expected from mere passive diffusion from circulating blood. This has been primarily evaluated as a diagnostic tool for herpes simplex encephalitis, and there are reports supporting its use in poliomyelitis (Openshaw and Lieberman, 1983), zoster encephalitis (Andiman et al. 1982), and Epstein-Barr virus encephalitis (Joncas et al. 1974). At present,