Aspects of Slow and Persistent Virus Infections

edited by
D. A. J. Tyrrell

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Clinical Research Centre, Harrow

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PREFACE

This book records the papers and discussions at a Workshop which took place in London on the 5th and 6th of April 1979, as part of the programme of the Commission of the European Communities on Medical and Public Health Research. However the views expressed are those of the individuals concerned and not of the EEC or any of its organs. The object was to discuss certain biological aspects of natural and experimental slow virus infections. Because the amount of knowledge and the focus of interest varied in respect of each infection the approach and emphasis varied also. In the case of scrapie, we discussed the nature of the agent and the mode of pathogenesis, in the case of SSPE, the search for unusual features of the virus, and recent detailed work on the immunology of the disease. As for Visna we reviewed the present understanding of the virus and its pathogenicity and also field epidemiology and methods for its control. There were also general papers, on interferon and oncornaviruses for example. We thank all those who made the meeting possible and enabled us to produce this book quickly, so that those who could not attend the meeting may nevertheless be able to read a great deal of what went on at it. In particular we would thank the Ciba Foundation who allowed us the use of their premises and Mrs. Jean Ashley who dealt with most of the arrangements. Last but not least we thank Dr. R.N.P. Sutton who as supporter and discussion editor rapidly produced a summary of the discussion which took place.

D.A.J. Tyrrell

LIST OF CONTRIBUTORS

G. Agnarsdottir
The Royal Postgraduate Medical School
Departments of Immunology and Virology
Du Cane Road
LONDON, W 12
England

M. Bergeret
Institut National de la Santé et de la Recherche Médicale
u.-43, Hôpital Saint-Vincent-de-Paul
PARIS, 75014
France

G.F. de Boer
Centraal Diergeneeskundig Instituut
Afd. Virologie
Houtribweg 39
8221 RA LELYSTAD
The Netherlands

M.F. Bourgade
Institut National de la Santé et de la Recherche Médicale
u.-43, Hôpital Saint-Vincent-de-Paul
PARIS, 75014
France

C. Chany
Institut National de la Santé et de la Recherche Médicale
u.-43, Hôpital Saint-Vincent-de-Paul
PARIS, 75014
France

F. Chany-Fournier
Institut National de la Santé et de la Recherche Médicale
u.-43, Hôpital Saint-Vincent-de-Paul
PARIS, 75014
France

P.W. Ewan
The Royal Postgraduate Medical School
Departments of Immunology and Virology
Du Cane Road
LONDON, W 12
England

H. Fraser
ARC Animal Breeding Research Organisation
King's Buildings, West Mains Road
EDINBURGH, EH9 3JQ
Scotland

K.B. Fraser
Department of Microbiology and Immunology
The Queen's University of Belfast
Grosvenor Road
BELFAST, BT12 6BN
Northern Ireland

G. Georgsson
Institute for Experimental Pathology
University of Iceland
KELDUR, REYKJAVÍK
Iceland

N.T. Gorman
MRC Group on Mechanisms in Tumour Immunity
The Medical School
Hills Road
CAMBRIDGE
England

J. Habicht
MRC Group on Mechanisms in Tumour Immunity
The Medical School
Hills Road
CAMBRIDGE
England

D.J. Houwers
Centraal Diergeneeskundig Instituut
Afd. Virologie
Houtribweg 39
8221 RA LELYSTAD
The Netherlands

R.H. Kimberlin
Institute for Research on Animal Diseases
Compton, Near Newbury
BERKS, RG16 ONN
England

W. Kreth
Institut für Virologie und Immunologie
der Universitat Wurzburg
Versbacher Landstrasse 7
WURZBURG 8700
Germany

P. Lachmann
Addenbrookes Hospital
Hills Road
CAMBRIDGE, CB2 2QQ
England

E. Lund
Department of Veterinary Virology and Immunology
The Royal Veterinary and Agricultural
University of Copenhagen
13, Bulowsvej
1870 COPENHAGEN
Denmark

J.R. Martin
Institute for Experimental Pathology
University of Iceland
Keldur
REYKJAVÍK
Iceland

V. ter Meulen
Institut für Virologie und Immunbiologie
der Universtität Wurzburg
Versbacher Landstrasse 7
WURZBURG 8700
Germany

C.A. Mims
Department of Microbiology
Guy's Hospital Medical School
London Bridge
LONDON, SE1 9RT
England

N. Nathanson
Institute for Experimental Pathology
University of Iceland
Keldur
REYKJAVIK
Iceland

H. Pabst
Institut für Virologie und Immunologie
der Universität Würzburg
Versbacher Landstrasse 7
WURZBURG 8700
Germany

P.A. Pálsson
Institute for Experimental Pathology
University of Iceland
Keldur
REYKJAVIK
Iceland

A. Pauloin
Institut National de la Santé et de la Recherche Médicale
u.-43, Hôpital Saint-Vincent-de-Paul
PARIS, 75014
France

G. Pétursson
Institute for Experimental Pathology
University of Iceland
Keldur
REYKJAVIK
Iceland

D. Sergiescu
Institut National de la Santé et de la Recherche Médicale
u.-43, Hôpital Saint-Vincent-de-Paul
PARIS, 74014
France

J.R. Stephenson
Institute of Virology and Immunobiology
University of Würzburg
Versbacherstrasse 7
8700 WURZBURG
Germany

L.Thiry
Head, Department of Virology
Institut Pasteur du Brabant
Rue du Remorqueur 28
1040 BRUSSELS
Belgium

H. Valdimarsson
Department of Immunology
St. Mary's Hospital Medical School
Praed Street
LONDON W2
England

R.A. Weiss
Imperial Cancer Research Fund Laboratories
P.O. Box 123
Lincoln's Inn Fields
LONDON WC2A 3PX
England

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INTRODUCTION (C. A. MIMS)

There are two ways of looking at slow and persistent virus infections. The first is to consider their immense biological interest, whether or not they are of any practical importance. For instance, I could maintain that the most fascinating persistent virus of all is lactic dehydrogenase virus in mice. But this infection, in which only macrophages are involved and in which there are puzzling immunological phenomena, causes no pathological changes, no illness, and is of little or no importance for the mouse. The second approach to slow and persistent virus infections is a clinical one and reflects our eagerness to discover that viruses are behind this or that chronic disease of unknown aetiology. The neurologists, rheumatologists, and those who deal with cancer are interested from this point of view. The two approaches often overlap. SSPE, for instance, although it is a clinical problem, has a wider biological interest. We cannot understand oncornaviruses or visna virus without considering their relationship with the host genes and with the host species - in other words their general biology. Much of the scrapie work focuses unashamedly on scrapie as a fascinating problem in general biology but scrapie is also a practical problem in sheep.

It is a great pleasure to see that both the biological and the more clinical or practical aspects of these infections have been so neatly fitted into our programme. Its good also to see some immunology because immunology comes into everything, and you cannot understand any infectious process without looking at the immune response.

The rest of my short introductory talk consists of three points: First, there must be some more persistent viruses waiting to be discovered in man. I do not refer to C - type viruses, which for all I know have already been discovered in the form of nucleic acid sequences or virus - specific enzymes. But there are the papovaviruses, a fine set of persistent viruses, many of which are still what we used to call orphan viruses, looking for diseases. JC and BK viruses, excreted in the urine of transplant patients and pregnant women, infect most of us, and we need to learn more about

them. But there appear to be other human viruses in this group, because non - BK non - JC viruses have also been isolated. Even the common wart virus has now been unequivocally divided into at least four distinct types by restriction enzyme analysis. Dr. Kalder at the San Antonio Primate Centre now has seven antigenically distinct simian foamy viruses. Surely there are some human foamy viruses. If so, then it is possible they have no effects on their host, in which case their biological interest is great but their practical importance zero. Are there representatives of visna virus in man? Were the reports of visna antibodies in human serum false alarms?

The second point is that we may discover that some of the old viruses do unexpected things. If human picornaviruses are capable of persisting or remaining latent like Theiler's virus in mice, it will raise many possibilities. Chronic infection with Theiler's virus sometimes causes an immunologically mediated demyelinating disease in mice. There have been attempts to find poliovirus RNA sequences in amyotrophic lateral sclerosis, but so far these have been unsuccessful. Even C - type viruses can be neurotropic, and one of them causes a chronic neurological disease in the mouse, probably by a direct effect of the virus rather than via the immune response. Yellow fever virus may seem an odd one to mention at such a meeting, but I have noticed how difficult it is to explain to immunologists how neutralizing antibodies to yellow fever remain at high levels for 50 - 70 years after the primary infection, when the virus was presumably eliminated from the body. Could it be that in some corner of the lymphoreticular system viral antigens persist, or there is a very slow turn over of productive infection? Hepatitis B virus certainly persists, but little is known about its ability to infect or remain latent in parts of the body other than the liver.

My last point is about viruses and the immune system. This is an area of research which seems full of opportunities. If a virus is to establish a persistent infection it must come to terms with immune responses, either by-passing them, avoiding them, or inducing ineffective responses. It can be no accident that nearly all persistent viruses, and also scrapie, go first to lymphoid tissues. To evade host defences, what

more audacious but logical a strategy than to invade and in some way weaken these defences. There are various fascinating possibilities. We have suitable experimental techniques for dissecting out this interaction of viruses with lymphoreticular tissues, and by using the in vitro spleen cell system for instance it should be possible to discover a great deal that is relevant for persistence.

And now with great pleasure I will make way for those who have some hard data to present.

THE BIOLOGY OF SCRAPIE AGENT

H. KIMBERLIN

1. TRANSMISSIBILITY OF SCRAPIE

Scrapie is a fatal disease of the CNS that occurs naturally in sheep and goats (1). The clinical signs are variable but affected animals have either incoordinated movements, particularly in the hind limbs or show signs of intense pruritis. Commonly, both types of abnormality occur. The disease is diagnosed by clinical signs and the presence of vacuolated nerve cells in histological sections of brain. Interstitial spongy degeneration is often found in the same areas as neuronal vacuolation and occasionally there may be neuronal loss. Hypertrophy of astrocytes occurs as an additional but non-specific lesion. Demyelination is either very slight or absent and there are no inflammatory changes to indicate the presence of an infectious agent (Chapter 4 and reference 2).

However, there is no doubt that scrapie is caused by a transmissible agent. The injection of brain homogenates from affected sheep will transmit the disease to other sheep after long incubation periods which sometimes last for several years (1). The transmissible agent can be filtered (3,4) and experimentally passaged in sheep to extremely high dilutions of original inoculum (5) thus demonstrating the existence of a replicating, virus-like agent. Experimental forms of scrapie have been produced in many species (Table 1), notably mice and hamsters. It is important that several strains of mouse passaged agent have been injected into sheep and produced scrapie (7). As discussed later, scrapie is an infectious disease (section 3.3.1) and the causal agent shows the expected micro-biological properties of strain variation (section 3.4) and

mutation (section 3.7).

Table I. Known susceptible hosts for experimental scrapie

Group	Species
Ruminant	Sheep, Goat
Carnivore	Mink
Old World Monkey	Cynomolgous
New World Monkey	Squirrel, Capuchin, Spider
Rodent	Mouse, Rat, Gerbil, Vole
	Hamster (Syrian and Chinese)

Adapted from reference 6

2. PHYSICOCHEMICAL PROPERTIES OF SCRAPIE AGENT

Despite intensive study, there is little firm information on the nature of the scrapie agent (8). The only available assay is by titration in animal hosts, which even in the quickest model of scrapie (strain 263K in hamsters; 9) takes 150-200 days. Infectivity titres accurately reflect amounts of agent in inocula that are chemically similar but the proportionality between titre and agent changes when some chemical treatments are used, for example sodium dodecyl sulphate (SDS) (10). This happens because highly purified agent is not available and the non-scrapie components in a tissue extract may become chemically modified on treatment and, as a consequence, the efficiency of infection is altered. Hence much of the published data are difficult to interpret, particularly when infectivity titres differ by only 1 to 2 $\log_{10}$ LD_{50} units.

Most studies have been carried out with the 139A strain of mouse passaged agent or with other strains from the 'drowsy-goat' source. In retrospect this may have been a mistake because there are some indications that biologi-

cally different strains of agent have different physicochemical properties. For example, the inactivation of the 22C strain of agent was about 3 $\log_{10}$ LD_{50} units greater than that of the 22A agent when 10 percent saline homogenates of scrapie mouse brain were autoclaved at 110°C for 30 minutes (11). Because of these findings it may be premature to draw general conclusions about the nature of the scrapie agent.

Another limitation of past work is that most of it has been carried out with scrapie brains taken in the clinical stage of the disease. Table 2 shows the results of three preliminary experiments on the effects of SDS on titre in scrapie brains taken at different times during incubation. There is a clear pattern showing an apparent increased inactivation of scrapie (strain 139A) at earlier times than at later times. This pattern could be due to structural differences between early and late synthesised agent or, alternatively, to an alteration in brain tissue as lesions develop in the second half of the incubation period.

With these limitations in mind, the following is a brief summary of the main findings on the nature of the 139A (Chandler) strain of agent. In general the agent is highly stable when exposed to many physicochemical treatments, for example wet heat, alkylating agents, organic solvents, concentrated salt solutions and many detergents (8). This stability is probably related to the common finding that infectivity is functionally associated with cell membranes particularly in the microsome fraction. In one study of the SMB cell line (12), derived from a scrapie-affected brain and persistently infected with agent, the highest infectivity titres were found in the plasma membrane of the cell. Treatments which disaggregate membrane structures, e.g. 80% 2-chloroethanol, 90% phenol, 5% SDS (8,13), also appear to destroy most of the scrapie infectivity, again suggesting a link between agent and membranes. The agent has not been identified by the

Table 2. Effect of SDS on scrapie infectivity in brain homogenates prepared at different times in the incubation period

Days after i.c. infection with strain 139A	Infectivity titres ($-\log_{10}$ i.c. LD_{50} units/.03g)			
	Agent titre in brain	Loss of titre after treatment with SDS		
		Expt. 1	Expt. 2	Expt. 3
35	5.25		$\geqslant$ 2.50	
46	6.17	2.17		
49	6.21		2.14	
64	6.50		2.25	
68	7.27			3.25
76	7.00	1.17		
96	7.29			2.61
112	7.50		1.56	
117	7.33	1.00		
126	7.88		1.88	
138	7.88			2.41

Pooled mouse brains were homogenised in 0.32M sucrose at a concentration of 10% w/v and centrifuged at 1,000g for 10 min. to remove nuclei, myelin and unbroken cells. The supernatants were further centrifuged at 100,000g for 1 h. to sediment particulate material and most of the scrapie infectivity. The pellets were resuspended in saline at a concentration equivalent to 10% whole brain and aliquots were incubated with equal volumes of 1% SDS at pH 8.4. In experiments 1, 2 and 3, incubations were carried out at 20°C for 1 h., 37°C for 1 h. and 37°C for 2 h., respectively. Titrations of infectivity were performed on serial ten-fold dilutions injected intracerebrally (i.c.) into Compton white mice. Unpublished data of Kimberlin and Walker.