SLOW VIRUS INFECTIONS OF THE CENTRAL NERVOUS SYSTEM

Edited by
Volker ter Meulen
and
Michael katz

SLOW VIRUS INFECTIONS OF THE CENTRAL NERVOUS SYSTEM

Investigational Approaches to Etiology and Pathogenesis of These Diseases

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VOLKER ter MEULEN / MICHAEL KATZ

WITH 97 ILLUSTRATIONS

Springer-Verlag
New York Heidelberg Berlin

Proceedings: Workshop on Slow Virus Infections:
University of Würzburg: March 24–26, 1975

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Library of Congress Cataloging in Publication Data

Workshop on Slow Virus Infections, University of Würzburg, 1975.
Slow virus infections of the central nervous system.

Includes bibliographical references and index.
1. Virus diseases, Slow—Congresses. 2. Nervous system—Diseases—Congresses. I. ter Meulen, Volker, 1928- II. Katz Michael. III. Title
RC114.6.W67 1975 616.8'04'194 77-1570
ISBN 0-387-90188-4

9 8 7 6 5 4 3 2 1

ISBN 0-387-90188-4 Springer-Verlag New York

ISBN 3-540-90188-4 Springer-Verlag Berlin Heidelberg

Preface

This book is a result of a Workshop held at the Institute of Virology of the University of Würzburg, Germany in March of 1975. The Workshop was organized to bring together investigators of slow virus infections and other scientists, who have not engaged in such research, but who were leaders in the field of biologic investigations. The conveners hoped that these latter would be more objective critics, who would express their views freely and who would offer recommendations for new directions and new approaches to the investigations of slow virus infections. These expectations were fulfilled as the organization of the Workshop permitted much free time for discussion as well as time for directed critiques. This volume represents an extensive summary of the proceedings and the discussions that followed. We believe it will be of interest to all investigators of slow virus infections and to the students of host-parasite relationships.

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PART I
UNCONVENTIONAL AGENTS

CHAPTER 1

Scrapie: Pathogenesis in Inbred Mice: An Assessment of Host Control and Response Involving Many Strains of Agent

A. G. DICKINSON / H. FRASER

INTRODUCTION

Scrapie is a fatal progressive degenerative disorder of the central nervous system that occurs as a natural infection in sheep and goats. It is transmissible experimentally to various species, including mice, but the disease has not been produced in all species that have been tested, such as, rabbits and guinea pigs. There are many strains of the agent that causes scrapie, and their molecular structure is probably outside the range for conventional viruses. Consequently, they display very high resistance to inactivation by a wide range of physical and chemical treatments. Although scrapie agents remain infectious after treatment with very large doses of 254 nm UV irradiation, this finding does not necessarily exclude nucleic acids as the informational molecule in scrapie, as many have assumed. It is possible that nucleic acids can be protected chemically, or repaired, in ways not yet known. Because of these uncertainties many workers have preferred to use the operational term "agent" rather than "virus."

The many unusual properties have prompted various hypotheses about the nature of these agents, though none of them can now accommodate all the findings. An unfortunate corollary has been the uncritical acceptance of various unlikely "findings" that would quickly be recognized as experimental errors, if conventional microorganisms were involved. The only agreed remnant of these hypotheses is that most of the infectivity, detectable by present methods in brain homogenates from mice with advanced disease, accompanies cell membranes, and it is assumed that this association is present in the living animal. It is unknown whether this applies to most of the agent earlier in incubation or in tissues other than brain. Neither the form of this association with membranes nor its significance, if any, for agent replication or protection is known.

Infectivity assays in whole animals are the only available tests for the presence of the agent: There are no immunologic, electron microscopic, or tissue culture findings on which to base *in vitro* tests. Many attempts have

been made to grow tissues (usually brain) from affected animals in which the agent could be shown to be replicating, but there has only been satisfactory evidence of success in one case (2).

AGENT REPLICATION

Agent Replication during Incubation in Mice

The importance of the lymphoreticular system (LRS) in the early pathogenesis of scrapie first became apparent from a time-sequence study involving 13 organs (14). The same general pattern appears to be followed by various agent strains, at least in the most widely used mouse strains, but the different host/agent combinations vary widely in the absolute time intervals involved.

The sequence of events follows. The earliest rise in titer occurs in organs of the LRS, such as the spleen, after either intracerebral (i.c.) or extraneural injection. Agent titer rises at a later stage in other tissues, such as the lungs, the intestines, and the uterus, but only later, if the extraneural injection in the spinal cord and brain led to death after a relatively short clinical course. It is unknown how the agent is transported from the site of injection to the LRS and, with peripheral injections, eventually from the LRS to the brain. No convincing "viremic" phase has been detected, with the exception of the brief one occurring immediately after i.c. or intravenous injection. Presently, it is reasonable to assume that rise of titer in an organ indicates that replication is occurring there. In the case of an i.c. injection, the extraneural events seem to be irrelevant to the course of the disease because the agent that remains in the brain starts to replicate there much sooner than it would have after extraneural injection, though still not as soon as it does in the spleen. It is possible that the agent that has received some types of treatment (e.g., heating) (7, 13) may not do so, but with ordinary inocula, there are several reasons for concluding that the agent replicates in the brain after an i.c. injection: Incubation is shortest after i.c. injection, even when sterile i.c. injection-trauma accompany intraperitoneal (i.p.) injection of the agent; splenectomy (or genetic asplenia) has no effect after an i.c. injection but increases incubation after an i.p. injection (8, 18); the effective titer of an inoculum is higher by the i.c. than the i.p. route (Fig. 1.1); neonatal and young mice are easier to infect by the i.c. than the i.p. route (27); the *supression* of susceptibility with large doses of steroids does not apply to i.c. injections.

Replication in the spleen can occur fairly quickly. It is possible that there is a short delay of a day or so before replication commences in the spleen in the quicker host/agent combinations (e.g., ME7 agent in C57BL mice, with 160-day incubation period after an i.c. injection of 10^5 LD_{50} units). But this then proceeds with a doubling time of about 2 days, and the process is complete 4 to 5 weeks later. Afterward, there is a titer plateau phase for the remaining 17 weeks before death. The amount of agent dur-

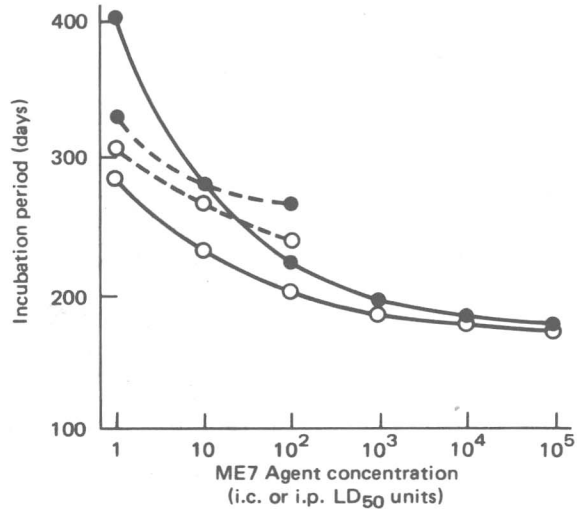

Figure 1.1.
Dose response curves for ME7-infected brain homogenate from BALB/c mice, titrated i.c. (solid line) or i.p. (broken line) in BALB/c mice (closed circles) or BRVR mice (open circles). Log_{10} LD_{50} estimated titers per 2 milligrams of brain: i.c. in BALB/c, 6.5; i.c. in BRVR, 6.6; i.p. in BALB/c, 4.0; i.p. in BRVR, 4.1.

ing this plateau is only about 1 i.c. LD_{50} unit/50 spleen cells, but in the brain the concentration of agent eventually reaches about one infective unit per cell. It is unknown whether the infectivity is evenly distributed between cells or highly concentrated in a few of them.

Various procedures can interfere with these events, but they all relate to the earliest phase of the process. It seems possible to reduce the effective susceptibility of mice to infection either physiologically, pharmacologically, or genetically, and these appear to act by preventing infection from taking place or by delaying the start of replication. Once replication has commenced, it appears to proceed inexorably. It must be emphasized that there is no evidence that scrapie is ever present as a latent infection of a type that can be activated by random events within the host or the environment.

Effects on Agent Replication

With all the mouse-passaged agents that have been tested, there is a *precise* inverse relationship between dose and incubation period, but the absolute dose/time details depend on the particular mouse strain/agent strain combination and the route of injection (Figs. 1.1 and 1.2).

The limitation imposed by only having one method of assay is not always appreciated: We have no means of knowing how much agent is functionally present in a tissue—functional, that is, for replication of more agent, or "stored" but able to produce damage—we can only estimate an operational titer and *assume* that it bears a direct relationship to the functional concentration. Another independent method of assay could give some perspective on the validity of this assumption, and an indication of the uncertainty of the present position is given by the fact that assays by i.p. injection give estimates 10 to 500-fold lower than by the i.c. route and that assay in different strains of mice using the same route can differ to a similar extent (5, 11).

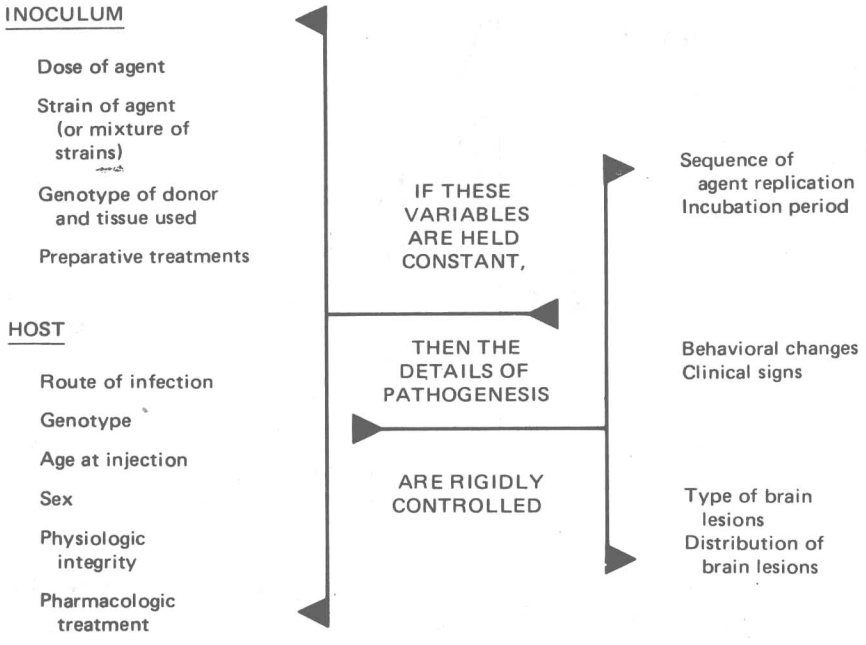

Figure 1.2.
Principal sources of variation in the pathogenesis of scrapie.

The Maturation of Susceptibility

The effect of age at injection on an incubation period in mice differs according to the route of injection. With i.c. injections there is a slight decrease in incubation with increasing age from birth to, at least, halfway through the lifespan (e.g., with ME7 in C57BL a decrease of about 1 day/20 days of age). Also with i.c. injections there is no indication that susceptibility varies with age, but this is not so with i.p. injections, when the younger mice are *less* susceptible.

The maturation of susceptibility appears to follow the same time course as the maturation of immune responses, which become well developed in the second postnatal week. Given the extreme stability of scrapie agents, it is surprising that all or most of the infectivity in the inoculum does not persist in the young mouse for the few days before the animal becomes susceptible. Very large doses of the agent do appear to persist and can produce an unexpectedly short incubation. The infectivity in intermediate and lower doses, however, appears to be quickly removed, even though it is unknown whether this is done by excretion or, more probably, by some degradation processes. It is easy to see that these could be "swamped" by very large doses of the agent. It seems reasonable to expect that these processes also occur in the older mice and that the maturation of susceptibility does not mean that they cease, but that they are involved in the development of readily accessible cellular sites where the agent can be protected from degradation and can, as a result, replicate.

Treatment of mice with prednisone acetate tends to restore the neonatal

state of insusceptibility (28), but as age increases, larger or repeated doses of prednisone are needed for this effect. It is unlikely that this effect is the result of T-cell suppression because adult thymectomy (plus irradiation) does not affect incubation after i.p. injection (24). However, the general feature of scrapie presented so far makes some other types of cells in the LRS an attractive possibility for agent replication. Treatment of mice with arachis oil has an effect similar to, but less dramatic than, those of prednisone (29). It remains to be determined whether the oil acts because of its unsaturated fatty acids or because it contains traces of potent anti-inflammatory substances.

Agent Strain Variation and Strain-Typing Techniques

A common strategic mistake has been to use only those combinations of agent strain, mouse strain, and route of injection that give the shortest incubation period as a basis for generalizations about "scrapie in the mouse." There is great variation in the timing of events during incubation between different strains of the agent and also between mice differing in their alleles of the gene *sinc* (abbreviation for *s*crapie *inc*ubation), which controls the incubation period.

It is unfortunate that most work with scrapie has stemmed from sources (SSBP/1 sheep or "drowsy" goats) that contain several agent strains and that the majority of natural cases of scrapie in sheep appear to be infected with more than one strain (6). In retrospect, it can be seen that claims about modification of "the agent" by passage in different species can be explained by recognizing that the starting material was a mixture of strains that become more homogeneous on passage, due to the new hosts restricting replication of some of them.

As a consequence, it is no longer possible to interpret gross agent changes due to passage history in terms that imply modification of the structure of the infective units, unless it can be established that the starting material was a homogeneous agent strain. Cloning of agents, by serial passage in inbred mice from end-point dilution cases is the only practicable starting point for critical studies. Even with such cloned agents, the possibility of "mutation" (or whatever the scrapie equivalent is) has to be taken into account, because roughly 2^{20} replications occur between injection and death and there is no information on the frequency of replication errors.

We have developed various agent-typing techniques during the last decade, and they have recently been employed to probe some of the molecular events in agent replication and its control by the host. Although recent results have shown that strains of agents can differ in thermal stability—22C being much more labile than 22A (13)—all the basic techniques for identifying agents stem from the differences in pathogenesis. These are of two types, which are largely independent of each other: tests that use the absolute and relative differences in the speed of incubation in a standard array of inbred and F_1 mice (4, 5, 12) and tests that use quantitative and qualitative aspects of the brain lesions (15–17, 19).