CULTURE OF RABIES VIRUS IN VITRO

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ABSTRACT

Since the early 1950s, cell culture systems have been developed which have led to the understanding of much of the structure and biology of rabies virus and have also made it possible to grow the virus in sufficient quantities for vaccine production. In this chapter, we give a brief historical account of the production of the wide variety of cell systems available today and of how they have been employed in the various areas of research and virus assay. The information gained in terms of virus infection and pathogenesis in vivo is discussed, as are the ways in which cell cultures are now applied in the important areas of diagnosis and epizootiology of the disease. There is a short résumé on vaccine production and the problems of transferring the appropriate technology to the developing countries. We conclude by considering the many questions that remain and how tissue culture techniques may assist in providing some of the answers.

INTRODUCTION

Rabies virus (RV) is remarkable in its apparent ability to infect and kill all mammalian species. The virus is regarded as highly neurotropic, yet in the infected animal after replication in the central nervous system it spreads centrifugally to most organs in the body, in which it is often able to replicate efficiently. It is not surprising, therefore, that in vitro rabies virus can grow in a wide variety of cells (see reviews 1-3). Indeed, much of the information contained in this book could not have been obtained had cell culture techniques been unavailable.
HISTORY

Attempts to grow RV in vitro date from before World War I when Noguchi (4) and Levaditi (5,6) independently reported the prolonged release of virus from cultured fragments of nervous tissue taken from infected animals. No further attempts to culture the virus were reported until after 1930 when it was shown that fixed RV would replicate in both mouse and rat embryo brain cultures and tumor cells (cited in refs. 2,3).

The susceptibility of primary mouse kidney cell cultures to RV infection was reported by Vieuchange and coworkers in 1956 (7,8). Two years later, Kissling described the successful serial passage of both street and fixed virus in primary hamster kidney cells (9), and by 1963 Kissling and Reese (10) had demonstrated the potential use of virus grown this way for the preparation of a vaccine. It has since been shown that large scale production of RV is also possible in a variety of primary culture systems including monkey (11), dog (12) and pig kidney (13), and chick embryo (CEF) and duck embryo fibroblasts (14).

Nowadays, there are a number of cell lines and cell strains available for the regular production of large quantities of RV (2,3). Such cell lines include BHK-21 (15), Nil-2 (16), CER (17) and Vero (18). Neuroblastoma cell lines of mouse (19-22) and human (3,21) origin are very susceptible to RV infection, and are frequently used in diagnostic tests and for the study of virus virulence (19-22).

Because of their heteroploid characteristics and oncogenic potential, none of the cell lines, with the exception of Vero cells (18,23), has been considered suitable for the production of human vaccines. However, in 1964 Wiktor and coworkers (24) described the production of vaccine in a human diploid cell strain, WI-38. Other human diploid cell strains such as HEL (25) and MRC5 (26) have been used for the same purpose, and a rhesus monkey diploid cell line vaccine has also been produced (27,28).

The propagation of RV has also been demonstrated in a number of cell lines of poikilothermic origin (2,3) but not in insect cells (29). Other unusual cells, e.g. embryonic chick myotubes (30) and a mouse macrophage cell line (31) have also been used for in vitro studies of RV infection and pathogenesis.