The Epstein – Barr Virus

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With 72 Figures and 29 Tables

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Preface

The Epstein-Barr virus was discovered 15 years ago. Since that time an immense body of information has been accumulated on this agent which has come to assume great significance in many different fields of biological science. Thus, the virus has very special relevance in human medicine and oncology, in tumor virology, in immunology, and in molecular virology, since it is the cause of infectious mononucleosis and also the first human cancer virus, etiologically related to endemic Burkitt's lymphoma and probably to nasopharyngeal carcinoma. In addition, continuous human lymphoid cell lines initiated and maintained by the transforming function of the virus genome provide a laboratory tool with wide and ever-growing applications.

Innumerable papers on the Epstein-Barr virus have appeared over recent years and reports of work with this agent now constitute a veritable flood. The present book provides the first and only comprehensive, authoritative over-view of all aspects of the virus by authors who have been the original and major contributors in their particular disciplines.

A complete and up-to-date survey of this unique and important agent is thus provided which should be of great interest to experts, teachers, and students engaged in cancer research, virology, immunology, molecular biology, epidemiology, and cell culture. Where topics have been dealt with from more than one of these viewpoints, some inevitable overlap and duplication has resulted; although this has been kept to a minimum, it has been retained in some places because of positive usefulness.

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Key to Abbreviations

ADLC	antibody-dependent lymphocytotoxicity
B cell/lymphocyte	bone marrow-derived lymphocyte
BL	Burkitt's lymphoma
BUDR	5-bromodeoxyuridine
CF	complement fixing/fixation
CMV	cytomegalovirus
CPV	carp pox virus
DEAE	diethyl-aminoethyl-dextran
EA	early antigen
EA (D)	early antigen (diffuse)
EA (R)	early antigen (restricted)
EAV	equine abortion virus
EBNA	EBV nuclear antigen
EBV	Epstein-Barr virus
EMA	early membrane antigen
GPHV	guinea pig herpesvirus
HSV	herpes simplex virus
HVA	herpesvirus ateles
HVP	herpesvirus papio
HVS	herpesvirus saimiri
HVT	herpesvirus of turkeys
IBR	infectious bovine rhinotracheitis virus
IF	immunofluorescence
IM	infectious mononucleosis
IUDR	5-iododeoxyuridine
LA	late antigen
LCL	lymphoblastoid cell lines
LHV	Lucké's herpesvirus
LMA	late membrane antigen
LYDMA	lymphocyte-detected membrane antigen
MA	membrane antigen
MATSA	Marek's disease tumor-associated surface antigen
MDV	Marek's disease virus
MPMV	Mason-Pfizer virus
NPC	undifferentiated nasopharyngeal carcinoma
PFU	plaque forming units
p. i.	post inoculation

PRV	pseudorabies virus
T cell/lymphocyte	thymus-dependent lymphocyte
TD_{50}	dose of virus transforming 50% of a series of cultures
UV	ultraviolet
VCA	viral capsid antigen
VZV	varicella-zoster virus

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1 Introduction: Discovery and General Biology of the Virus

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A. INTRODUCTION

By the beginning of the 1960s viruses causing tumors in animals had been known for more than 50 years (Gross, 1961), but at that time there was no such agent with a convincing etiologic link to human cancer. Since then a remarkable association has been uncovered between a new herpesvirus and malignancy in man; the association has been established by work of many different kinds, but the original discoveries which provided the impetus for this work resulted from experiments undertaken because of clinical and epidemiologic observations on an unusual lymphoma of children in Africa.

The first account of this lymphoma (Burkitt, 1958) attracted little attention. However, in March 1961 Denis Burkitt, then an unknown surgeon working in Uganda, addressed a staff meeting at the Middlesex Hospital Medical School, London (Fig. 1), and presented for the first time outside Africa his pioneer studies which seemed to show that the distribution of the tumor was determined by geographic features affecting climate. That the incidence of what soon came to be known as Burkitt's lymphoma (BL) was influenced by temperature and rainfall suggested at once that some biologic factor was concerned, and of these a climate-dependent arthropod vector seemed the most likely. If an insect or other arthropod were indeed spreading the tumor the further important implication followed that a transmissible agent such as a virus must be involved in causation. It was this exciting hypothesis which was directly responsible for our immediate decision to seek for possible oncogenic viruses in BL material.

A COMBINED MEDICAL AND SURGICAL STAFF MEETING

will be held

on Wednesday, 22nd March, 1961 at 5.15 p.m.

IN THE COURTAULD LECTURE THEATRE.

Mr. D.P.Burkitt from Makerere College,
Uganda will talk on "The Commonest Children's
Cancer in Tropical Africa. A Hitherto
unrecognised Syndrome".

Fig. 1. Photograph of the original notice announcing a staff meeting at the Middlesex Hospital, London, at which Burkitt gave the first account outside Africa of the clinical and epidemiologic features of the lymphoma which now bears his name. From Epstein, M. A.: Long-term tissue culture of Burkitt's lymphoma cells. In: Burkitt's lymphoma. Burkitt, D. P., Wright, D. H. (eds.). Livingstone, 1970: Courtesy of the editors and publisher

Although the original concept of case-to-case infection mediated by a climate-dependent arthropod vector (Burkitt, 1962a, b) required revision as epidemiologic information accumulated (Haddow, 1964), other explanations for the role of temperature and rainfall have emerged (Burkitt, 1969) and the idea of an infectious cause has remained constant (Chap. 14).

B. DISCOVERY OF EBV

I. PRELIMINARY INVESTIGATIONS

As part of the various investigations on BL undertaken in our laboratory at the Middlesex Hospital Medical School, virologic studies were of prime importance. BL biopsy samples were deep-frozen immediately after removal and were flown overnight from Uganda to London. On arrival the tumor material was thawed and prepared in many different ways for inoculation into newborn mice, embryonated hen eggs, and test tissue culture systems in order to isolate any viruses which might have been present. However, these early experiments proved uniformly negative and thin sections of tumor samples were therefore searched in the electron microscope in an effort to find unusual viruses which might not be demonstrable by standard biologic isolation procedures; such direct examinations were likewise negative.

It was then considered (Epstein et al., 1964a) that success might be achieved if BL cells from biopsy samples transported at room temperature could be grown in vitro away from host defences so that an otherwise inapparent oncogenic virus might be able to replicate, as happens with cultured cells from certain virus-induced animal tumors (Bonar et al., 1960). Because of this, it was decided that in the further search for virus in BL, high priority should be given to the establishment of lines of the tumor cells capable of growing in continuous long-term culture, even though the prospects for accomplishing this with a lymphoid tumor were unpromising. For, at that time, no member of the human lymphocytic series of cells had been grown as a permanent line in vitro despite repeated efforts ever since the earliest phases of the tissue culture technique (Woodliff, 1964).

II. CULTURE OF BURKITT'S LYMPHOMA CELLS AND FINDING OF THE VIRUS

After a long series of trial methods a successful procedure for culturing BL tumor cells was evolved in the latter part of 1963 and the EB1 line of continuously growing BL-derived lymphoblasts was established (Epstein and Barr, 1964); a full account of both the preliminary trials and the definitive culture technique was given the following year (Epstein and Barr, 1965).

As soon as sufficient material could be spared from the first EB1 cultures, electron microscopy was undertaken; pelleted cells were examined in thin sections, virus particles were observed in a cell within the very first grid square to be searched, and the virus was immediately recognized as a morphologically typical member of the herpes group (Epstein et al., 1964b). When first seen there was naturally no means of knowing which herpesvirus was involved, but it was thought unusual that a member of the herpes family was being carried as an inapparent infection in a continuous human cell line without causing destruction of the cultures. Preliminary biologic tests for herpesviruses were applied to the virus-bearing EB1 cells using embryonated hen eggs, HeLa cells, and young mice inoculated intracerebrally, but in each case the results were negative. When further extensive virologic investigations were also negative (Epstein et al., 1965) it became obvious that the agent was unlike any known herpesvirus since it showed a complete lack of biologic activity in any of the standard test systems. With