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Beneficial Effects of Human Viruses

GEORGE MILLER, M.D.,[a] AND DUNCAN K. FISCHER[b]

[a]*Departments of Pediatrics, Epidemiology and Public Health, and* [b]*Molecular Biophysics and Biochemistry, Yale University School of Medicine, New Haven, Connecticut*

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In keeping with the theme of this Yale-China symposium, we discuss some unexpected dividends which have been derived from the basic study of five viruses to which man has been exposed. Inquiring into the behavior of these viruses for their own sake has not only produced an increase in basic understanding of biologic processes, but has provided concepts and techniques which will broaden our knowledge of the etiology, pathogenesis, and treatment of human diseases which are unrelated to viruses.

INTRODUCTION

In this paper we shall attempt to summarize some unexpected ways in which human viruses have proven to be beneficial to their natural host—man. The concept of "beneficial human viruses" represents a recent change in the thinking of microbiologists about their subjects. From an initial preoccupation with microbes as etiologic agents in infectious diseases, microbiologists are now focused on ways in which microorganisms can instruct them in the nature of basic biologic processes and on efforts to use microorganisms to produce interesting and potentially useful macromolecules.

Medical virology was initially the domain of clinicians and researchers who wished to prevent viral disease. The beneficial effects to man of their efforts cannot be overstated. The isolation of the etiologic agents of smallpox, poliomyelitis, measles, yellow fever, rubella, and mumps, and the preparation of effective, easily administered vaccines against these classical epidemic viral diseases of man surely rank among the great triumphs of public health. Nevertheless, definite challenges remain in answering numerous perplexing questions. How exactly did these efforts, based largely on the empirical efficacy of vaccination with attenuated live viruses, succeed in conferring protection against later infection with the true viral pathogen? Moreover, what type and degree of protection was conferred to the vaccinated subject and specifically how did the mediators of host resistance act? For despite the eradication of smallpox from the world and the near total elimination of poliomyelitis from our country, we still have only vague and untested ideas of how such avirulent live virus vaccines work. More specifically, we do not know why wild-type poliomyelitis virus occasionally affects certain cells in the nervous system or why attenuated vaccine

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Address reprint requests to: George Miller, M.D., Department of Pediatrics, Yale University School of Medicine, 333 Cedar St., New Haven, CT 06510

virus almost always fails to do so. The irony is that these avirulent strains are beneficial agents to man only because they are antigenically related to viruses which are definitely harmful.

This lack of fundamental knowledge about the basic mechanisms involved in the life cycle of viruses has led to intense scrutiny of the biology and molecular biology of all classes of human viruses. We will provide five examples of the ways in which such study has provided new concepts and methods.

SV_{40}—A MODEL FOR CANCER RESEARCH

Simian virus 40 was originally isolated as a contaminant from rhesus monkey kidney cells used for the production of early batches of poliovirus vaccines [1]. The natural hosts of SV_{40} are monkey cells in which a permissive or productive infection can proceed; however, when rodent or human cells which do not permit complete replication of the virus are infected, a fraction of the exposed cells becomes "transformed" into cells with properties of cancer cells.

The cell transformation phenomenon can be studied using the tools of molecular genetics, since viral DNA persists in the altered cell and integrates into the host genome. In the transformed cell only a portion of the integrated genome is transcribed into mRNA. This mRNA is encoded by the so-called "early region" of the genome. The gene products of this region are implicated in the transformation process, for mutations in the early region lead to loss of the ability of SV_{40} to induce or maintain the transformed phenotype [2].

The major gene products of the early region are two proteins, the tumor antigens, called big T (94 K MW) and little t (19 K MW). The large T antigen is known to have several interesting properties. It is a DNA binding phosphoprotein, which binds preferentially to the origin of DNA synthesis of the viral genome [3]. It stimulates both viral and cellular DNA synthesis [4]. It is closely associated with host cell proteins (host tumor antigens) which are present in a wide variety of human and nonhuman tumors [5].

Thus SV_{40} has provided us with a number of useful phenomena which will certainly clarify disease mechanisms. These include a reproducible way to change a normal human cell into a malignant one, a method of integrating foreign DNA into a normal chromosome, and the definition of one or more proteins which seem to affect cellular DNA synthesis.

The hope is that unravelling the mechanisms of oncogenesis by a simple virus such as SV_{40}, which contains only about 5,200 nucleotides, will provide insights which are generally applicable to other carcinogenic agents. The most optimistic idea is that various oncogenic agents, viral or otherwise, will follow a final common pathway.

ADENOVIRUSES—THE DISCOVERY OF RNA SPLICING

Adenoviruses are larger, more complex agents than SV_{40} for they contain about 20×10^6 Daltons of DNA (about seven times the amount in SV_{40}). These viruses cause a variety of different diseases of the respiratory tract of man, including conjunctivitis, pharyngitis, and pneumonia [6]. They establish latent infections in the lymphoid tissue (hence the name "adeno") of the nose and throat, and they can readily be recovered from tonsils and adenoids [7]. The role that these persistent viruses play in the genesis of disease has not been clarified.

Adenoviruses, like SV_{40}, cause transformation of cultured rodent cells. All serotypes of adenovirus are able to induce morphologic changes in tissue cultures, but only some serotypes are oncogenic in animals. Thus adenoviruses have made

clear that cell transformation studied *in vitro* and oncogenesis in the intact animal are distinct processes [8].

Apart from their utility as models for studying cancer, adenoviruses have played a major role in research on transcription of messenger RNA. In part, adenoviruses were destined for such a role since they can be grown in very large quantities in the laboratory in cell culture and because adenoviral infection of human cells results in a productive, lytic infection in which host cell DNA and RNA syntheses drop dramatically. Since host RNA synthesis is inhibited, viral message can be isolated and studied in relatively pure form. It was soon discovered by Darnell and his collaborators that very large adenovirus 2 (Ad 2) RNA species were found in the cell nucleus, but far smaller adenoviral mRNAs became associated with the cytoplasmic ribosomes [9]. This led to the idea of processing of the mRNA in which large nuclear RNA species are shortened in some fashion as they are being transported to the cytoplasm. Philip Sharp and his colleagues subsequently noticed that Ad 2 mRNAs contained sequences from sometimes widely separated regions of the genome [10]. The splicing process involves transcription of a large primary transcript with a retained 5′ leader sequence, the looping out and elimination of intervening non-mRNA sequences, and the ligation of the 5′ end with coding sequences downstream. The coding regions of the gene are called exons and the eliminated regions removed by the splicing reaction, introns. Thus the transcript of a gene may be much smaller in size than the gene itself.

RNA splicing, initially discovered in adenovirus, has turned out to be a general feature of the transcription of many, but not all, viral and cellular genes. For example splicing has been demonstrated to occur in the synthesis of mRNAs coding for hemoglobins and immunoglobulins [11,12]. Improved understanding of the thalassemias and of a variety of immune deficiency states will be achieved as the result of the discovery of splicing.

HERPES SIMPLEX VIRUS—TECHNIQUES FOR GENE TRANSFER

Herpes simplex virus (HSV) causes a number of human diseases such as herpes labialis, herpes genitalis, encephalitis, and disseminated neonatal infection [13]. The virus remains latent in the sensory trigeminal or sacral ganglia and can be periodically reactivated. The herpes simplex genome is a very large and complex molecule containing over 100 million daltons of DNA [14]. One of its genes specifies an enzyme thymidine kinase (TK), which catalyzes the phosphorylation of pyrimidine nucleotides. The TK enzyme is not essential for replication of herpes simplex virus *in vitro* but it appears to be needed for infection *in vivo* and for the establishment of latency [15].

Normal cells also contain a TK enzyme, which is distinguishable from the viral enzyme on the basis of several properties. Mutant cells which do not express the TK enzyme can be isolated by growth in the presence of bromodeoxyuridine (BrdU) [16]. Cells which become resistant to BrdU do not possess TK activity which is needed for the phosphorylation of the drug and its incorporation into DNA where it exerts its lethal effect. Selective media are also available in which cells lacking TK will not grow [17].

Several years ago it was discovered that the herpes virus TK enzyme could be used to convert cells which lacked a TK enzyme into ones which expressed it [18]. The experiments were first done with inactivated virus and later with intact viral DNA [19]. Recently transfer of the viral TK gene has been accomplished with a small fragment of herpes viral DNA which has been cloned on a bacterial plasmid. During

the course of studies on this form of "biochemical transformation" or gene transfer, Wigler and his colleagues made a surprising finding. Cells which took up the herpes DNA fragment and became stably transformed also took up other DNA molecules, regardless of their specificity. Thus by using the TK selection system, it became possible to transfer other eukaryotic genes [20]. Recently Ruddle and his colleagues at Yale have transferred the HSV TK genes to mouse eggs by microinjection, and they have raised mouse embryos which contain the herpes viral TK gene [21].

Viruses will increasingly be used to bring about gene transfer in human cells. It is now possible to incorporate a gene which specifies globin into an SV_{40} DNA molecule. Following infection of tissue culture cells with this recombinant DNA, the cells make globin [22]. Gene transfer experiments are used to define the functions of various regions of cellular and viral DNA and will ultimately be used as therapy for genetic deficiencies.

PARAINFLUENZA VIRUS AND CELL FUSION

Parainfluenza virus, which causes croup in children, has proved to be a valuable tool in development of genetics of eukaryotic cells. The Sendai strain of parainfluenza virus is highly potent in its ability to cause cells of different species to fuse and form polykaryons (or polynucleate cells) and subsequently to become heterokaryons and hybrid cells [23]. Fusion is brought about by a component of the Sendai virus envelope. In mouse-human hybrid cells, human chromosomes are randomly lost and mouse chromosomes selectively retained until the heterokaryon becomes stable. By generating a large number of clonal hybrid lines, each containing a different subset of the human karyotype, and by comparing which proteins these hybrid lines express with their chromosomal composition, an impressive number of genes have been localized or mapped to specific human chromosomes [24]. Cell fusion mediated by Sendai virus has given way to chemical cell fusion techniques, particularly by polyethylene glycol, but this virus opened a whole new field of somatic cell hybridization and its application in deciphering the genetics of eukaryotic cells.

Cell fusion also forms the basis of the so-called hybridoma method of producing monoclonal antibodies [25]. This technique involves immunizing a mouse with a partially purified or even complex antigen. The spleen cells are removed and fused with a myeloma cell line, which provides signals for continuous cell growth *in vitro* and possibly also for the secretion of large amounts of immunoglobulin. Selection is applied against the myeloma line which has been made deficient in thymidine kinase and thereby unable to grow in the HAT (hypoxanthine-aminopterin-thymidine) selective medium. Therefore, only the hybrid spleen/myeloma cells can grow. These are diluted in microwells so that each microwell initially contains one cell, and is then permitted to grow into mass culture. The fluid in each individual microwell is screened for the presence of the desired antibody. Since each microwell was seeded with one cell and according to immunologic theory one cell makes one antibody, the secreted antibodies are "monoclonal."

Recently fusion hybrids between human lymphocytes and a human myeloma line have been produced [26]. This should allow the production of human monoclonal antibodies which will be useful in a great many areas of medicine. For example, human monoclonal antibodies may be used to prevent specific diseases in situations where immune globulins prepared from human serum are now used, such as prophylaxis against chickenpox, hepatitis, or Rh-incompatible hemolytic disease. Furthermore, human monoclonal antibodies will be useful in the study of a variety of autoimmune diseases. It should be possible, for example, to make monoclonal

antibodies which represent the spectrum of autoimmune responses of patients with systemic lupus erythematosus.

THE EPSTEIN-BARR VIRUS AND LYMPHOID CELL LINES

Epstein-Barr virus, the etiologic agent of infectious mononucleosis, has the ability to cause certain human lymphocytes to grow indefinitely in the laboratory under tissue culture conditions, a process often referred to as immortalization [27]. The target cell for EBV is a bone marrow-derived (B) lymphocyte [28]. The B lymphocyte of any individual can be immortalized; without the addition of EBV, human lymphocytes will remain alive for weeks to months, but they will not divide and will eventually die off. The B lymphocyte immortalization capability of EBV has numerous potential applications and benefits.

Lymphoblastoid cell lines remain more or less stable in their chromosome number and other genetic characteristics, particularly during the first year of their *in vitro* life. Thus EB virus can be used to establish cell lines from patients with a wide variety of genetic disorders, such as abnormalities in hemoglobin synthesis, in immunoglobulin production, or disorders of DNA synthesis and DNA repair [29]. The biochemistry of such defects can be analyzed in lymphoblastoid cells, much as they have been analyzed in the past in skin fibroblasts from such patients. However, lymphoid cell lines are easier to establish and grow more readily.

Lymphoblastoid cell lines express histocompatibility antigens on their surface; in fact, the immortalized cells seem to express considerably more HLA antigens than do fresh, or primary lymphocytes. Thus EBV provides the material for isolation, purification, and biochemical characterization of different HLA antigens [30].

Certain lymphoblastoid lines secrete appreciable amounts of interferon into the culture medium. There is a current renaissance of interest in the use of human interferon as an antiviral agent; interferon also has cytolytic activity against certain tumor cells. One pharmaceutical company has begun the commercial production of interferon from large vats of a lymphoblastoid cell line called Namalwa [31]. Questions of safety of interferon produced by EB virus-transformed cells have been raised; however, the other method of production of human interferon, namely induction of interferon synthesis in different batches of fresh human leukocytes by Sendai virus or Newcastle disease virus, also carries the risk of induction of a variety of different latent viruses from different leukocyte donors. Interferon purification has progressed to the point where lymphoblastoid interferon would seem to be a safe product. Interferon produced by bacteria containing recombinant DNA would now seem to be the most likely source for clinical trials.

Recently Schlossman and colleagues at Harvard have put EBV to use in an ingenious experiment. They were interested to know whether there were different classes of T, or thymus-derived lymphocytes in man, as have been described in mice. They encountered a patient with an unusual form of chronic lymphocyte leukemia derived from T cells. They reasoned that this T cell leukemia was monoclonal, i.e., derived from just one type of T cell. They raised an antiserum in a rabbit against the leukemic cells. Then, to remove any activity which such an antiserum might have against B lymphocyte surfaces, they established a B lymphocyte cell line from the same patient by the use of EBV. The B lymphocyte line was then used to absorb the antiserum, leaving a specific anti-T serum. Further experiments with this anti-T serum showed that it reacted only with certain types of T lymphocytes and not others. Thus they had begun the subclassification and separation of human T cells into helper and suppressor categories [32].

B lymphocytes immortalized by EB virus are capable of immunoglobin (Ig) synthesis. Our colleague Dr. Nathaniel Brown and others have shown that EBV transformed cells can be cloned so that they produce only one type of heavy chain and one type of light chain; that is, monoclonal immunoglobulin [33]. Some of these clones secrete appreciable amounts of Ig into the culture fluid and in others the Ig remains intracellular. The question now arises whether EBV can be used to establish clonal B lymphocyte lines which are secreting a specific antibody. George Klein and co-workers have made preliminary reports of human antibody made by lymphoblastoid cell lines obtained from lymphocytes of persons sensitized against a chemical hapten [34]. Thus one possible future beneficial effect of EBV might be in the *in vitro* production of human monoclonal antibody without the need for fusion to a myeloma cell line.

These five examples of beneficial human viruses illustrate the way in which practical benefits and basic research are inexorably intertwined. Many more beneficial effects of viruses remain to be uncovered. However, these future benefits will probably not come as the result of highly goal-oriented task forces or special programs. Rather they are likely to be uncovered as small surprises which come unanticipated during the course of basic research done for its own sake.

Bibliographic Note

The references cited are not meant to convey priority, nor are they considered to be complete. They merely serve as access to selected areas of literature.

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