Meetings

Serially Cultured Animal Cells for Preparation of Viral Vaccines

At a meeting in Zagreb, Yugoslavia, held on 1 and 2 October 1968 under the auspices of the Yugoslavian Academy of Sciences and Arts, three problems were considered, based on data from several laboratories on the propagation of human diploid cell populations for use in preparing viral vaccines for man. Scientists from Belgium, Great Britain, France, the United States, the U.S.S.R., West Germany, and Yugoslavia participated.

The first problem discussed was the spontaneous transformation of the host cells. During serial propagation mammalian cells follow one of two general paths. After a varying number of cell generations the growth rate becomes progressively slower, and eventually the cells die (phase III phenomenon) or undergo spontaneous transformation. Characteristics of these transformed cells are varied and include indefinite survival in serial culture; a decreased susceptibility to contact inhibition of growth and division, with the result that cells pile up in a disordered pattern and reach much higher population densities per unit of surface area; decreased serum requirement for growth; the ability to multiply in soft agar or in fluid suspension; karyotypic changes of varying extent; and, most important, the ability to produce cancers in an appropriate experimental animal.

Cells of different mammalian species vary widely in the frequency with which they undergo spontaneous transformation in culture and in the stage of growth at which it occurs. Whereas human cells rarely transform, mouse cells do so regularly, and monkey and hamster cells transform occasionally. Studies by Gartler suggest the possibility that some or all spontaneous transformations of human cells result from contamination of the cultures by HeLa cells.

Although the multiple changes in these transformed cells are closely associated, they are not invariable. Thus, not all spontaneous transformants have been shown to be capable of producing tumors.

It is generally agreed that virally transformed cells should not be used for vaccine production. Since spontaneous transformants. Most of these tured from cancer tissue resemble virus transformants in many respects, including their ability to produce tumors in experimental animals, it is possible that some of them may also have been transformed by viruses in the course of their serial propagation; consequently, they, too, should probably not be used in the production of vaccines. Thus, the prevention of spontaneous transformation, as well as viral contamination, in candidate cell strains for use in vaccine production is of major importance.

The second problem discussed was the presence of oncogenic viruses. Oncogenic viruses may transform cultured cells with phenotypic consequences similar to those observed in many spontaneous transformants. Most of these viral transformants are aneuploid; most have escaped from contact inhibition of growth to a greater or lesser degree; most have a decreased serum requirement; and many (but not all) are capable of producing tumors upon injection into the appropriate host.

Among the oncogenic viruses demonstrated in primary cultures are SV40 and simian adenoviruses in monkey kidney, canine hepatitis virus in dog kidney, avian leukosis virus in chick embryo cultures, and murine leukemia in mouse cultures. So far, no oncogenic viruses have been demonstrated in either primary or serially propagated human cell cultures derived from organs suitable as a source of cell cultures for use in vaccine production.

The presence of contaminating nononcogenic viruses or other organisms such as mycoplasmas was the third topic. Yet another complication in the use of primary mammalian cell cultures for the preparation of viral vaccines is the presence of contaminating nononcogenic viruses, which were either present in the original tissue or introduced in the course of serial propagation. Examples are foamy viruses in monkey kidney cultures and cytomegalo- and coxsackievirus in human kidney cultures. The presence of such nononcogenic viruses is usually recognized by their cytopathic effects. However, there have been several examples of viruses growing parabiotically in serially propagated cells with no readily demonstrated cytologic changes. Worth noting are cytomegalovirus in green monkey cells, herpes B in rhesus monkey cells, and "Marburg agent" in green monkey kidney cultures. In the last two cases the virus was first detected when laboratory personnel became fatally infected.

The detection of these contaminating viruses has involved a variety of techniques (for example, animal inoculation, hemadsorption, immunofluorescence); and the refinement of these techniques, along with the development of new procedures, will probably disclose the presence of hitherto unrecognized viral contaminants in many tissues and derived cell cultures now considered free of virus.

Analogous to the introduction of nononcogenic viruses into cultured cells is the introduction of mycoplasmas which, in recent years in the United States, has to a surprising degree involved a single species (*Mycoplasma hyorhinis*). Mycoplasma contamination is undesirable, more perhaps because its effects are not known than because of the known complications it introduces. Mycoplasmas are usually introduced into cultures along with bacteria, by breaks in aseptic technique. When the culture medium contains antibiotics, these may eliminate bacterial contaminants without affecting antibiotic-resistant mycoplasmas. Paradoxically, mycoplasma contamination can therefore best be prevented by culturing cells in media free of antibiotic and in which bacterial contaminants can be readily recognized.

In considering these three problems the following general principles for the selection of a diploid cell substrate for viral vaccines were suggested:

1) In selecting a new species for the establishment of a standardized diploid cell population, tissue from a large number of individuals of the same age should be tested in order to determine the average finite life, to observe karyotype stability, and to determine the frequency of spontaneous transformations in serial culture. If the frequency of transformations is high, cell strains from that species should not be used for viral vaccine productions.

2) The tissue of origin should be one in which contaminating viruses have not been demonstrated. The difference in this respect between monkey and human tissue is noteworthy. The disappearance of demonstrable viruses from serially propagated monkey cells does not necessarily preclude the persistence of at least portions of the contaminating viral genome. Tissue of fetal origin is probably preferable to postnatal tissue because the former has a lower risk of exposure to extraneous viruses.

There are several new techniques for better detection of latent agents. Immunofluorescence tests with homospecific serum and fluorescent immunoglobulin to given species can reliably be used to detect viral coat protein in cell substrates.

Electron microscopy is proving useful for detecting viruses in cells and should be used routinely. More experience is needed before the data can be interpreted fully, but appearance of any virus-like particles calls for further studies or exclusion of the cell population. To investigate a new cell substrate for the presence of incomplete viruses, the technique of cell fusion with normal and transformed cells can be used to detect the defective or activated viral genome.

3) Since potential tumorigenicity of new cell substrates is a problem, attempts should be made to obtain a more sensitive procedure to test for this property. It has been shown that treating mice with antiserum to lymphocytes has allowed unplanted HeLa cells to establish rapidly growing tumors. Greater experience with other malignant and transformed cells in mice and other animals treated with immunosuppressive agents as well as drugs is needed and may lead to the development of a useful screening test for cell substrates. Attention should be given to the number of cells required for the production of tumors, both by known transformants and candidate diploid strains. With the latter, relatively large inocula should be used.

4) An important feature of the acceptability of a candidate diploid cell strain for human viral vaccine production is the maintenance of a "normal" diploid karyotype, as opposed to the abnormal or pseudodiploid karyotype which appears in transformed or neoplastic cell lines of indefinite subculture potential. Many cultures of the candidate substrate must be examined in order to assess the range of "normal" random variation in the occurrence of chromosome aberrations in serial culture. The range of variation may be compared with that of leukocytes of the same species. By defining these limits, cell strains may then be judged for compatibility with a range of abnormal karyotypes seen in peripheral leukocyte cultures from normal control subjects of the same species.

5) It is self-evident that the usefulness of a given cell strain depends both on the spectrum of viruses which may be propagated and their yield. Human diploid cell strains present significant advantages over primary cells with respect to many of the points considered above. The following vaccines have been prepared in human diploid cells, and some have already been used on an extensive scale.

Poliomyelitis (oral). The Immunological Institute in Zagreb, Yugoslavia, has had extensive experience with this vaccine including over 1 million vaccinations and some follow-up studies. The vaccine has been licensed for general use in Yugoslavia. Workers in the Soviet Union have also prepared and tested on a large scale polio vaccine prepared in human diploid cells.

Measles (live, attenuated). It has been shown, again by the Immunological Institute in Zagreb, and by the Institute for Viral Vaccine Production in Moscow, that measles virus grows well in human diploid cells. The vaccine so produced has been licensed in Yugoslavia and the U.S.S.R. for parenteral use on the basis of its low reaction rate and high protective capacity against the disease. Approximately 500,000 people in the U.S.S.R. have been inoculated with vaccine produced in human diploid cells.

Adenoviruses. Human diploid cell strains are at present the only cells in which adenovirus vaccines may be prepared without the possible introduction of helper viruses or genomes that might hybridize with adenoviruses. In these cells high virus titers and attenuation can be achieved. In the United States 350,000 subjects have been given enteric-coated capsules containing adeno 4 vaccine prepared by Wyeth Laboratories (Radnor, Pa.), with no untoward effects reported.

Rubella. Although rubella virus grows in many different cell cultures, only virus grown in human diploid cells has yet been regularly effective on both parenteral and nonparenteral administration (as reported by workers at the Wistar Institute, Philadelphia).

Smallpox. Vaccinia virus grows well in human diploid cells, and a vaccine free from contaminating agents has been prepared in these cells. Early studies by the Institute of Immunology in Zagreb have shown a satisfactory clinical response.

Respiratory syncytial virus (RSV). Although only experimental work has been reported with RSV vaccine, the growth of this virus to high titer in human diploid cells suggests the feasibility of the use of this tissue in vaccine production.

Rhinoviruses. Many rhinovirus strains grow only in human diploid cells and a number of laboratories are working on the preparation of killed- and live-virus vaccines produced in these substrates.

Rabies. There is an urgent need for a highly antigenic rabies vaccine produced on a cell substrate free of nerve cells. One such vaccine prepared in WI-38 cells is now being tested.

Tick-borne encephalitis. Tick-borne encephalitis virus grown to high titer in human diploid cells has been used for the production of an inactivated vaccine and found to be antigenic in experimental animals (Institute for Virus Preparation, Moscow).

This rapidly accumulating evidence of safety, together with the theoretical advantages of vaccines developed in human diploid cell cultures, indicates that this cell system is often to be preferred as a substrate for viral vaccines.

The conclusion of this conference is similar to that of a previous one on the same subject held in Bethesda, Maryland, in November 1967 (*National Cancer Institute Monograph No. 29*, Cell Cultures for Virus Vaccine Production, p. 583).

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