PRODUCTION OF INTERFERON IN HUMAN CELL CULTURES BY A NEW, POTENT VIRAL INDUCER

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Abstract

A newly discovered double-stranded RNA inducer of interferon, bluetongue virus (BTV), stimulates the production of large amounts of interferon in animals as well as in many types of mammalian cell cultures, including human leukocytes, and continuous cell lines. The exceptional pH lability of BTV and its lack of pathogenicity for man further recommend its use as an interferon inducer. Among several human cell lines tested, the most efficient producer of interferon was a continuous cell line designated HT-1376, derived from a bladder carcinoma. With infectious BTV as the inducer, the HT-1376 line produced more interferon per cell than did leukocytes; interferon yields ranged from 10,000 to 60,000 units per ml of crude, unconcentrated supernatant fluid. Noninfectious BTV, inactivated by ultraviolet irradiation, was as effective as infectious virions. The interferon produced in HT-1376 cells has physicochemical and antigenic properties resembling those of fibroblast interferon produced in diploid cells.

Introduction

Recent results of limited clinical studies (1, 2, 3) and considerable experimental evidence (4, 5) attest to the potential value of interferon in the treatment of human viral diseases and cancer. Cantell and his colleagues have developed a very good method using a viral inducer to produce potent leukocyte interferon in quantities sufficient for such clinical trials (6, 7, 8, 9). Because of the encouraging results obtained with that interferon, it appears that the needs for larger quantities of interferon may
be growing faster than the means of supplying it. There is thus increasing need for convenient, efficient, and economical methods to produce large quantities of interferon of consistent potency, stability, and purity.

Strander et al. have stated that the production of leukocyte interferon "... will always be limited by the availability of human blood" (10). The demand for leukocyte interferon may be moderated to some extent by a preference for interferon of fibroblast origin for some applications in cancer therapy. Such needs may develop, for example, from the recent observations that various types of tumor cells may respond better to the anticalcular action of the fibroblast interferon species (11). Available methods for the production of potent fibroblast interferon employ the superinduction process (12, 13, 14, 15), which may require minor adjustments, such as modification of actinomycin D concentration, for each cell strain (J. Vilcek, personal communication, 1977) and may pose problems for use on a large scale. Since the improvements being made in interferon purification methods should permit its separation from inducers and cell culture by-products, all available means of production should be considered.

To obtain a reproducible system for the production of large quantities of interferon, we have searched for (i) a continuous cell line capable of producing high yields of interferon, and (ii) a viral inducer that would be highly effective in human cells. The cell lines tested for interferon production were selected on the basis of cell type, availability, and growth potential. The prospective value of any given cell was estimated by its response to at least two different inducers, and interferon yields were compared with those obtainable with lymphocyte cultures. Three inducers were selected on the basis of their proven effectiveness in other cells (7, 16): polyriboinosinate-polyribocytidylate complexed with DEAE-dextran, Sendai virus, and Newcastle disease virus (NDV). A fourth, bluetongue virus (BTV), a member of the orbivirus subgroup of the double-stranded RNA-containing reovirus family, is the most potent interferon inducer in mice we have found, consistently producing more that 100,000 units/ml in plasma after injection of $10^8$ PFU/mouse (17,18). BTV was consistently an excellent inducer in rabbit kidney cell cultures, producing an average of 40,000 units/ml in more than 20 different experiments; these yields made it possible to produce the high-titered reference interferon standard G019-902-528 for the National Institutes of Health. BTV has two other properties which add to its suitability as a desirable interferon inducer: (i) its extreme pH lability (19) permits total inactivation of residual virus within one hour of exposure to pH 2, and (ii) the virus has no known human pathogenicity (20); D. W. Verwoerd, personal communication, 1976) a safety factor to be considered in large-scale production.