Isolation and Some Serological and Epidemiological Data on the Viruses Recovered from Patients with Subacute Thyroiditis de Quervain

D. Stanček 1, M. Stančeková-Gressnerová 2, M. Janotka 3, P. Hnilica 4, and D. Oravec 5

1 Regional Hygiene Station, Regional Institute of Health, 2 Chair of General and Applied Virology, Faculty of Natural Sciences, Comenius University, 3 Divisions of Radioisotopes, Department of Medicine, Medical Faculty Hospital, 4 Postgraduate Medical Institute, Department of Medicine, 5 Endocrinological Station, Regional Institute of Health, Bratislava

Received January 9, 1975

Abstract. Virological and serological methods were used in examination of 28 patients suffering from subacute thyroiditis de Quervain. Attempts to isolate a presumed viral agent from 8 patients were performed by inoculation of serum, urine, and aspiration biopsies of thyroid glands taken at different stages of the illness, into tissue cultures of different types of human and animal cells. Recovery of a cytopathic viral agent on cells of a rabbit lung continuous line was successful in 5 cases. Serological cross reactions exist between the isolated viruses and patient serum but not with serum of healthy people. Cases with the acquired illness and positive antibodies against the isolated viruses who had been in close and prolonged contact with patients suffering from subacute thyroiditis de Quervain were also investigated.

Introduction

Inflammatory infections of thyroid gland are not a rare form of pathological disturbance in this organ. They have often occurred in limited epidemics (Green, 1971; Janotka et al., 1974) or following acute inflammatory infections of the upper respiratory tract such as mumps, measles, infectious mononucleosis, influenza, and others (Werner and Ingbar, 1971). It is presumed that their incidence has shown a tendency to increase in the last years (Woolner et al., 1962). The etiology of these inflammations, however, remains an unresolved question. Among infectious agents, viral origin of the thyroid gland inflammations has only been studied rarely, though viruses are often suspected to be responsible for the acute or more or less prolonged inflammatory affections of this organ. Paramyxoviruses, such as the mumps virus, have often been considered to have a causal relationship with thyroiditis de Quervain (Eylan et al., 1957). Other reports pointing to an etiological relationship between myxo- or paramyxoviruses and subacute thyroiditis are based on indirect, mostly serological or epidemiological, data (Volpé et al., 1967; Felix-Davis, 1958). In one case of chronic thyroiditis and thyroid carcinoma, herpes-like particles and particles resembling oncogenic-type C viruses were seen by electronmicroscopy in a buffy-coat cell line derived from a patient (Maruyma et al., 1968).
By inoculation of rabbit lung cells with materials from a patient suffering from subacute thyroiditis de Quervain we succeeded at the end of 1972 in isolating a so far unclassified virus (Stanček and Gressnerová, 1974). In the present report we describe further results of our virological, serological, and in part epidemiological studies concerning the etiology of subacute thyroiditis de Quervain.

Materials and Methods

Tissue Cultures. Rabbit lung cell cultures (RLC) (Szanto, 1960) were supplied by the tissue culture laboratory of the Institute of Virology, Slovak Academy of Sciences. The cells were cultivated in Earle's medium enriched with glucose, yeastolate, 5 to 10% heat-inactivated calf serum (SEVAC, Prague), and antibiotics. The usual concentration of cells grown in Roux bottles or glass tubes was about $2 \times 10^8$ cells per 1 ml of media. A permanent line of hamster kidney cells (BHK), also supplied by the tissue culture laboratory of the Institute of Virology, was cultivated in Eagle's Basal Medium, supplemented with 10% of heat-inactivated calf serum and antibiotics. The cell concentration was the same as that with RLC. Cultures of human diploid cells obtained regularly from the Institute of Sera and Vaccines in Prague were kept in EPL medium (SEVAC, Prague) with 10% calf serum and antibiotics.

Titration of the Isolated Viruses. The viral isolates adapted to RLC cultures were titrated either by inoculation of newly transferred cultures of RLC or by inoculation of the infectious materials on 2 to 3 day-old monolayers of RLC; 3 to 4 glass tube cultures were always inoculated with one dilution of virus. The infected cultures were incubated at 35°C or 37°C for 3 to 5 days. Results were read according to the cytopathic effect (CPE) of the virus consisting of syncytia formation which, at a sufficient multiplicity of infection, were large and well separated, resembling plaques. The reciprocal of highest dilution of the inocula giving rise at least to 2 to 3 syncytia in each of the parallel cultures was considered to be the titer.

Virus Neutralization Tests. 0.4 ml of patient serum or control serum diluted 1:2 or 1:10 were mixed with 0.4 ml of one of the isolated viruses designated as the “MGI strain”. The titer of the virus was about 400 CPD$_{50}$ per 0.4 ml. The mixture was incubated at 35°C for 90 min. After incubation 0.2 ml of the mixture was transferred into tubes with $8 \times 10^4$ to $1.2 \times 10^8$ RLC. The treated cultures were then incubated at 35°C or 37°C. At the same time uninfected control cultures and cultures infected with the same dose of the virus used in neutralization tests were prepared. Results were usually read between the 4th and 8th days of incubation. Calculations were done by comparing the number of “plaques” formed by syncytia in the infected control cultures and the cultures treated with mixtures of serum and virus. Only those sera were considered positive which in a given dilution neutralized at least 50% of the control “plaques”. We counted average amounts of plaques in 5 to 10 microscopical fields of each of the 2 to 4 parallel cultures.

Indirect Immunofluorescence. 1 to 5-day-old RLC cultures infected with MGI strain or uninfected control cultures grown on cover slips (8 x 20 mm) placed in glass tubes were rinsed with buffered saline solution, pH 7.2 (BSS), dried and fixed in distilled acetone for 10 min at room temperature. The fixed cover slips were used either immediately or stored at $-20^\circ$C for several weeks. Fixed cells were rinsed before use with BSS and 0.1 to 0.15 ml of serum diluted 1:2 or 1:10 in BSS was added. The cultures were then incubated for 45 min at 35°C in a moist chamber. After thorough washing in BSS, cultures were treated with 0.1 ml of fluorescein-labeled swine globulin against human globulin (SwAHu/FITC, SEVAC, Prague) and kept for 45 min at 35°C in a moist chamber. After staining, the cultures were washed 3 times for 20 min in BSS and once in redistilled water. Finally, cover slips were mounted in glycerol-Tris buffer solution pH 8.0. Fluorescent microscope Fluoval, Carl Zeiss, Jena, was used for observation of results.

An indirect immunofluorescent test with sera to test for the presence of antibodies against Epstein-Barr virus capsid antigen (EB-VCA) was carried out according to the method described by Henle et al. Lymphoblastoid cell lines derived from patients with Burkitt's lymphoma were kindly supplied by Dr. J. Werner from the Robert Koch Institute in West Berlin.