ANTIBODIES TO STRUCTURAL AND NONSTRUCTURAL GAG-CODED PROTEINS OF TYPE D RETROVIRUSES IN PATIENTS WITH LYMPHADENOPATHIES

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Type D retroviruses are replicatively normal viruses associated with certain normal and tumor cells of primates; they do not induce cell transformation either in vivo or in vitro [6]. Several type D retroviruses are etiological agents of AIDS in monkeys, a disease which resembles in its course and manifestations human AIDS [5, 7]. However, viruses causing AIDS in man and type D retroviruses causing AIDS in monkeys are unrelated [4].

Type D retroviruses with minor differences from the prototype of this group of viruses, namely Mason-Pfizer monkey virus (M-PMV), were isolated from several transplantable HeLa-like human cell lines. It was shown previously that the primary translation products of the gag genes of type D retroviruses from transplantable human HEp-2 cells and of cells from monkeys infected with M-PMV virus are polyproteins with equal molecular weight (78 kD) [3, 9], which later separate into internal structural proteins. Two other gag-coded polyproteins have been discovered in HEp-2 cells producing type D retroviruses; both were glycosylated — gPr78 and gPr90. These same polyproteins were found on the plasma membrane of HEp-2 cells [1].

Considering association of type D retroviruses with human cells and their ability to induce T-cell immunodeficiency in monkeys, we have looked for antibodies to structural and nonstructural proteins of type D retroviruses in patients with lymphadenopathies (one manifestation of immunodeficiency). Radioimmunoprecipitation (RIP) and immunoblotting methods were used.

EXPERIMENTAL METHOD

Experiments were carried out on transplantable human laryngeal carcinoma cell line HEp-2 (producing type D retroviruses), cultured on a liquid nutrient medium of the following composition: 45% medium 199, 45% lactalbumin hydrolysate, bovine serum 10%. A transplantable cell line CV-1 of green monkey kidney was cultured on liquid nutrient medium RPMI-1640 with the addition of bovine serum. Commercial preparations of Mason-Pfizer virus (M-PMV, from CMMT cells), obtained from the USA in connection with Soviet-American collaboration on Oncovirology, were used. Commercial serum against p27 (the principal internal protein) of M-PMV was obtained from from the USA under similar arrangements. A preparation of commercial normal human plasma was used. Individual sera of patients with lymphadenopathies were obtained from the Children's Polyclinic of the All-Union Oncologic Scientific Center, Academy of Medical Sciences of the USSR, and some sera were provided by Dr. Med. Sci. V. N. Stepina (All-Union Oncologic Scientific Center, Academy of Medical Sciences of the USSR). The cells were transferred to Hanks' salt solution 2 h before introduction of the radioactive label. ^{35}S-methionine in a concentration of 50 μCi/ml (specific activity 18.5 GBq/mmole; from Amersham International, England) was introduced in Hanks' solution for 1 h. The medium with label was then removed, the cells washed 3 times with Hanks' solution, and then removed mechanically from the glass. The cells were lysed as described previously [1] and the lysate centrifuged at 10,000g for 45 min. The clarified lysate was used for RIP. To 70-80 μl of lysate 5 μl of the control or test serum was added, followed 1 h later by 30 μl of a 10% suspension of *Staphylococcus aureus* to coarsen the precipitate. Incubation with *Staph. aureus* was carried out for 16 h at 4°C. The precipitate was washed as described previously [1] and treated with 3% sodium dodecylsulfate (SDS; final concentration), with 5% 2-mercaptoethanol in 0.01 M Tris-HCl (pH 6.8), and with

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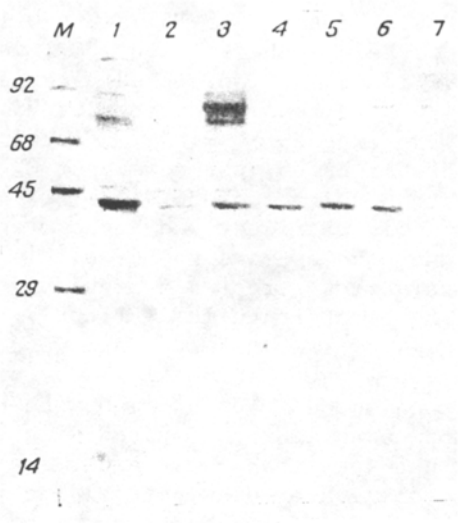

Fig. 1

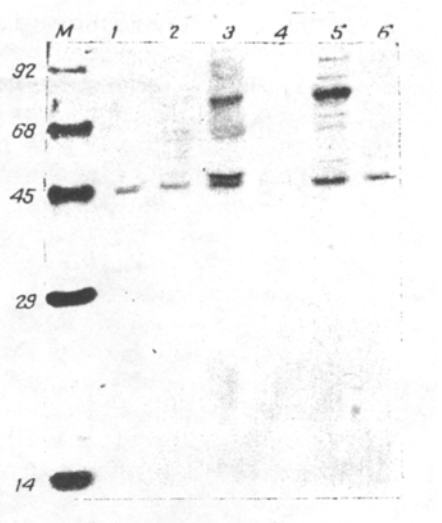

Fig. 2

Fig. 1. Search for antibodies to nonstructural gag-coded proteins of type D retroviruses in patients with lymphadenopathies and AIDS by the RIP method. 1, 2) Lysate of CV-1 cells, precipitated by anti-p27 M-PMV (negative control); 3, 7) lysate of HEp-2 cells precipitated by anti-p27 M-PMV (positive control); 4) lysate of HEp-2 cells, precipitated by normal human plasma (negative control); 5) lysate of HEp-2 cells, precipitated by serum from patient with lymphadenopathy; 6) lysate of HEp-2 cells, precipitated by serum of patient with AIDS. Here and in Fig. 2: M) ^{14}C-labeled molecular weight markers; position of $Pr78^{gag}$ indicated by arrow.

Fig. 2. Discovery of antibodies to nonstructural gag-coded proteins of type D retrovirus in patients with lymphadenopathies. 1-4) Lysate of HEp-2 cells, precipitated by individual patients' sera; 5) lysate of HEp-2 cells, precipitated by anti-p27 M-PMV (positive control); 6) lysate of HEp-2 cells precipitated by normal human plasma (negative control).

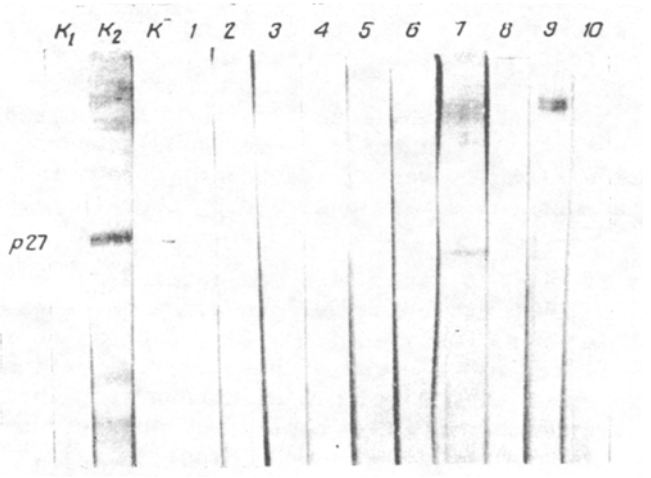

Fig. 3. Discovery of antibodies to structural gag-coded proteins of M-PMV in patients with lymphadenopathies. K_1) Filter treated with anti-p10-12 M-PMV (positive control); K_2) filter treated with anti-p27 M-PMV (positive control); K) filter treated with normal human plasma 1:100 (negative control); 1-10) filters treated with individual patients' sera (dilution 1:100). Position of p27 (principal internal protein of M-PMV) indicated on strips.

10% glycerol, heated at 100°C for 3 min, centrifuged at 4000g for 10 min, and applied to polyacrylamide gel (PAG). Electrophoresis was carried out in a PAG gradient (7.5-15%) with 0.1% SDS in the buffer system suggested previously [8]. A set of proteins labeled with ^{14}C (Amersham International) and also a set of SDS-6H marker proteins (Sigma, USA) were used as molecular weight markers. Fluorography was done by the method in [2]. To record the results HS II x-ray film (OR WO, East Germany) was used. The fluorograms were exposed at −70°C. Proteins were transferred to a nitrocellulose filter (Schleicher und Schüll BA 85, pore diameter 0.45 μ) in a modified GD-4 gel decolorizer (Pharmacia, Sweden), with a voltage of 36 V for 3.5-4 h, after preparative electrophoresis. After transfer, a narrow strip of filter was cut off and stained with 0.1% Amido Black 10B in 7% acetic acid, and the unbound dye was washed off in 7% acetic acid. The remainder of the filter was then cut into narrow strips 4-5 mm wide, which were incubated in a 3% solution of ovalbumin in 10 mM Na-phosphate buffer (PB; pH 7.4). The strips were then washed 5 or 6 times with PB containing 0.1% of Triton X-100, and were then incubated with individual sera from patients, diluted 1:100-1:400. Incubation was carried out for not less than 4 h on a shaker. The filter was then washed 5 or 6 times with PB with Triton X-100 and the strips incubated with antibodies and human IgG, conjugated with horseradish peroxidase (Amersham) in a dilution of 1:500 for not less than 3 h. After repeated washing with PB the strips of filter were placed in a solution of diaminobenzidine (0.5 mg/ml in 50 mM Tris-HCl, pH 7.5), to which 0.001% H_2O_2 had been added. The reaction developed in the course of 5-10 min.

EXPERIMENTAL RESULTS

It was shown previously that the formation of internal structural proteins of type D retroviruses (HEp-2 and M-PMV) takes place as a result of cleavage of a precursor polyprotein with mol. wt. of 78 kD [3, 9]. This polyprotein is a complex antigen carrying several (but not all) antigenic determinants of all the structural proteins contained in it. It thus appeared worthwhile to use this polyprotein to reveal the different antibodies to individual internal structural proteins of type D retroviruses. Some of the available sera from patients with lymphadenopathies also were tested by the RIP method, in which polyproteins (Pr78) from the lysate of pulse-labeled HEp-2 cells were used as antigens.

The results of testing the patients' sera are given in Fig. 1. Lysate of labeled CV-1 cells (not producing type D retroviruses, precipitated by anti-p27 M-PMV (tracks 1 and 2) and lysate of labeled HEp-2 cells, precipitated by normal human plasma (track 4) were used as the negative control. Lysate of labeled HEp-2 cells precipitated by anti-p27 M-PMV (track 3) was used as the positive control. A positive reaction was observed in track 6. The results obtained during one such investigation are illustrated in Fig. 2. For instance, the results of precipitation of virus-specific proteins by sera from patients with lymphadenopathies are shown in tracks 1-4. Track 5 is the positive control and track 6 the negative control (precipitation with normal human plasma). In one case (track 3) a strong positive reaction was observed (a positive, but significantly weaker reaction is shown in track 2). Altogether 18 sera of patients with lymphadenopathies were analyzed by the RIP method and six of them reacted with $Pr78^{gag}$.

A more extensive study of the patients' sera was carried out by the immunoblotting method. As antigens proteins of the M-PMV preparation were used. Altogether more than 80 sera from patients with lymphadenopathies were analyzed (Fig. 3). A clear-positive reaction with p27 M-PMV was observed when the filter was treated with serum from patient No. 7, whereas a weaker reaction was obtained by treatment of the filters with sera from patients Nos. 2 and 3. Of 80 sera from patients with lymphadenopathies, in 12 cases antibodies to p27 M-PMV were found (the sera were tested in a dilution of 1:100-1:200). Incidentally, not all sera positive in RIP were positive also in the immunoblotting test, and vice versa. The majority of sera tested reacted with gp70 M-PMV, but because of the absence of an adequate positive control, the corresponding conclusions could not be drawn.

Thus type D retroviruses are associated with human lymphadenopathies and they may perhaps play a definite role in the development of these diseases.

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