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IMMUNOPRECIPITATION OF HERPES SIMPLEX VIRUS POLYPEPTIDES WITH HUMAN SERA IS RELATED TO THEIR ELISA TITRE

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Summary. - Out of 485 human sera tested by neutralization and enzyme immunoassay 13 were negative in both tests, while a positive correlation was found in 457 samples (94.3 %). The rest of sera (3.2 %) showed discordant results. Selected sera were examined, in addition, by Western blot and immunoprecipitation assays with the aim to analyse the precipitation profile of discordant sera and correlate the ELISA titre with the precipitation profiles. High titre sera precipitated the main glycoproteins (gC, gB, gE, gD), the capsid polypeptides (VP 5, VP 19, VP 21, VP 22) and several other structural and nonstructural proteins. The ability of sera to precipitate viral structural proteins was related to their ELISA titre rather than to their neutralizing activity.

Key words: Enzyme-linked immunosorbent assay; neutralization test; herpes simplex virus type 1; Western blot; immunoprecipitation

Introduction

Enzyme-linked immunosorbent assay (ELISA) has become the most powerful tool for viral antibody detection since its introduction for herpes simplex virus (HSV) antibody titration (Voller *et al.*, 1976). Several approaches have been adopted during the last decade, the simplest being the three layer solid phase enzyme-immunoassay, in which the antigen is directly absorbed to the microplate. Although the quality of antigen used for coating seems essential, different procedures were used for antigen preparation ranging from a crude sonicated cell extract (Cremer *et al.*, 1982; Gilljam *et al.*, 1985), through solubilized detergent extracted membrane antigens (Jeansson *et al.*, 1983; Ashley *et al.*, 1985; Lehtinen *et al.*, 1985) to a sucrose gradient-purified virus

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suspension (Shillitoe *et al.*, 1983). We describe our experience with a simple sonicated crude cell antigen used along with a noninfected control cell antigen. The results of ELISA and neutralization tests (NT) were compared with the precipitation profiles of selected sera in Western blot analysis and radioimmunoprecipitation followed by polyacrylamide gel electrophoresis.

Materials and Methods

Sera. The sera were collected from schizophrenic patients in the Regional Psychiatry Hospital, Pezinok and from healthy donors (Blood Transfusion and Haematology Clinic, Bratislava). The age of the donors ranged from 20 to 70 years. All sera were stored at -20°C.

Antigens. Strain KOS of the herpes simplex virus (HSV) type 1 was used to prepare the antigens for ELISA, Western blot (WB) and radioimmunoprecipitation (RIP) analysis. For ELISA, Vero cells were infected at MOI of 1-2 PFU/cell, scraped off at 24 hr post-infection (p.i.), into hypotonic reticulocyte buffer pH 7.2, frozen and thawn and sonicated (3 times 20 sec, cooled on ice), and clarified by centrifugation. The supernatant was inactivated with formalin and dialyzed against phosphate buffered saline (PBS). The antigen was titrated in a 4 layered ELISA using swine anti-HSV-1 IgG coated microplates and its activity was expressed in antigen units per μg protein. For WB about 3-4 PFU of KOS virus was inoculated per Vero cell; at 24 hr p.i., the cells were washed with cold BPS, scraped off into Snowden's buffer (1.6 mmol/1 $MgCl_2$, 6 mmol/1 KCl, 10 mmol/1 TRIS pH 8, 1 mmol/1 DTT, 1 % NP-40, 0.5 % DOC, 0.5 mmol/1 PMSF). After 20 min extraction at room temperature, the cell debris were removed by centrifugation and the supernatant was stored at -70°C. For RIP the KOS-infected Vero cells (5 PFU per cell) were incubated for 15 hr in the presence of 185 kBq per ml of ^{14}C amino acids (labelled from 5-15 hr p.i.). The medium was decanted, cells washed in PBS and scraped off into a small vol of PBS, sedimented, treated with the extraction buffer (0.15 mol/1 NaCl, 1 % sodium deoxycholate, 1 % Triton X-100, 0.1 % SDS, 0.01 mol/1 TRIS pH 7.4, 0.001 mol/1 PMSF) for 60 min and centrifuged. The supernatant was stored at -70°C til use.

ELISA. Domestic 96-well immunoplates (UMG ČSAV Prague) were coated with the viral antigen (10 μg/ml, about 20 antigenic units) and/or control antigen (prepared from noninfected Vero cells as described above) using carbonate buffer pH 9.6. The wells were saturated with 0.5 % BSA, washed and then serum was added in increasing dilutions. After washing with PBS-Tween-20 the conjugate was added (SwaHuIg/PxSevac, Prague) diluted 1:1000. Following washing, the reaction product was visualized with orthophenylenediamine (OPD) in citrate buffer pH 5 in the presence of H_2O_2 for 30 min in the darkness. The reaction was stopped with 2 M H_2SO_4. The OD_{492} ratios in the wells coated with viral and control antigens exceeding 2.1 were calculated to reed the endpoints using a Dynatech minireader.

Western blots. The antigen was boiled for 2 min in SDS-electrophoresis sample buffer (Spear and Roizman, 1972) electrophoresed on an 8 % polyacrylamide gel. The slabs were transferred onto nitrocellulose filters in the blotting buffer (6 g TRIS-HCl, 28.85 g glycin, 0.4 l methanol, 2 g SDS, 1.6 l redistilled H_2O) under 150 mA and 40 V current in a blotting apparatus. The filter strips were incubated in the tested serum diluted in PBS pH 7.2 in the presence of milk powder for 2 hr under continuous shaking. After washing, the strips were treated with SwAHuIg/Px R-1:50 conjugate for 2 hr, washed, incubated in DAB solution for 3-5 min, washed and dried.

Immunoprecipitation and SDS polyacrylamide gel electrophoresis. About 50 μl HSV-1 antigen was incubated with 50 μl undiluted tested serum (or monoclonal antibody or immune rabbit serum) for 2 hr at 37°C then with 100 μl of 10 % suspension of formalin-inactivated *Staphylococcus aureus* for 30 min. After centrifugation, the pellet was washed 200 μl lithium chloride (3 times), boiled in electrophoresis sample buffer and after clarification subjected to electrophoresis (8 % polyacrylamide gel). The dried gel was exposed to X-ray Rapid film at -70°C for 21 days.

Virus neutralization test was performed with the KOS strain by incubation of 100 PFU virus-serum mixtures for 2 hr in the absence as well as presence of complement (properly diluted fresh guinea pig serum). The sera were inactivated before use. Endpoints were read according to obvious reduction of semiconfluent CPE in the Leighton tubes between incubation days 2–4 p.i.

Results

Comparison of virus neutralization tests and ELISA

Table 1 shows that the higher neutralizing antibody (NA) levels as detected in the presence of complement were in good agreement with the ELISA titres. The serum NA titres were scored as low in the range of 2–64 and high in the dilution range of 128–4,096. The ELISA titres were scored as low in the range of 160–5,120 and high between 10,240 to 160,000. In groups 1, 3 and 5 there was a good agreement in both tests (98.4 %). From these, both tests were negative in 13 cases (2.7 %). In four sera the NA levels were high when tested in the presence of complement, but low in its absence; the ELISA titres were relatively low (2,560–5,120). Three sera showed low ELISA titres in the absence of NA.

Western blot analysis of selected sera

Group 1 sera did not precipitate any virus-specific polypeptides. Group 2 sera

Table 1. Comparison of neutralization tests and ELISA to HSV-1 in human sera

NT titre	ELISA titre	Patient No.		Serum reaction type
negat (< 2)	negat (< 160)	13	2.7 %	group 1
negat	low (160–5,120)	3	0.6 %	group 2
low (2–64)	low (160–5,120)	1	0.2 %	group 3
low (2–64)	high (10,240–160,000)	8	1.6 %	group 4
high (128–4,096)	high (10,240–160,000)	456	94.1 %	group 5
high* (128–4,096)	low** (2,560–5,120)	4	0.8 %	group 6
Total		485		

* in the absence of complement the titre was low (not exceeding 64) but definitely positive
** definitely positives at dilutions 2,560–5,120

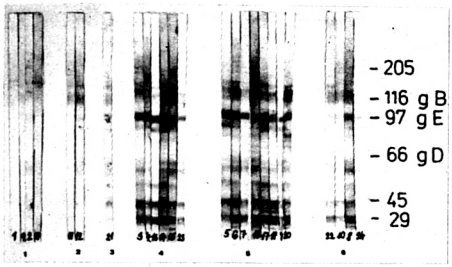


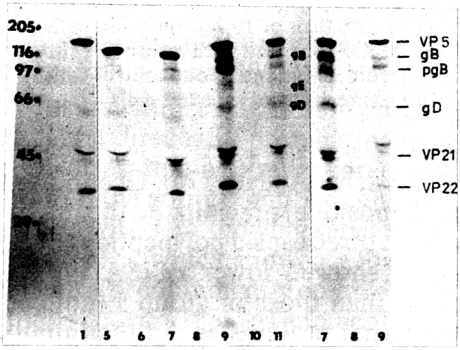
Fig. 1
Western blot with selected human sera against HSV-1 (KOS) antigen extracted from infected Vero cells
1 - lanes 1, 9, 2, 27 sera from group 1 (as indicated in Table 1).
2 - lanes 11, 12 sera from group 2
3 - lane 21 serum group 3
4 - sera from group 4 (lanes 3, 4, 13, 14, 15, 23)
5 - sera from group 5 (lanes 5, 6, 7, 16, 17, 18, 4, 20)
6 - sera from group 5 (lanes 22, 10, 8, 24)

precipitated two faint glycoprotein bands (gC and gB) and the main capsid polypeptide 155 kD (VP 5). Sera from groups 4 and 5 with ELISA titres higher than 5,120 precipitated at least 12 polypeptides, namely the main glycoproteins (gB, gC, gD and gE), several capsid polypeptides (VP 5, VP 19, VP 21, VP 22) and further structural and nonstructural virus-coded proteins (the completely negative reactions of the individual sera with the noninfected cell extracts are not shown). Group 6 sera with a relatively lower ELISA titre (2,560-5,120) precipitated about 10 polypeptides, but the bands were less intensive than those formed with the group 5 sera (Fig. 1).

Immunoprecipitation and immunoelectrophoresis

Fig. 2 shows the polypeptides precipitated by the sera of individual groups, as well as by immune rabbit serum and a monoclonal antibody to gB (Bystrická *et*

Fig. 2
RIPA-PAGE of ^{14}C-labelled KOS-infected and noninfected Vero cells with different antisera
A - 1 serum from group 1 (infected cells)
- 5, 6 serum from group 3 (infected and control cells); serum titres: NT,C+ 64; NT, C- 32; ELISA 2,560
- 7, 8 serum from group 4 (infected and control cells); serum titres: NT,C+ 256; NT,C- 64; ELISA 10,240
- 9, 10 serum from group 5 (infected and control cells); serum titres: NT,C+ 256; NT,C- 64; ELISA 81,920
- 11 serum from group 5 (infected cells only); serum titres: NT,C+ 256; NT,C- 64; ELISA 10,240
B - 7,8 immune rabbit serum against infected and control cells
- 9 ascitic fluid (monoclonal antibody) to gB



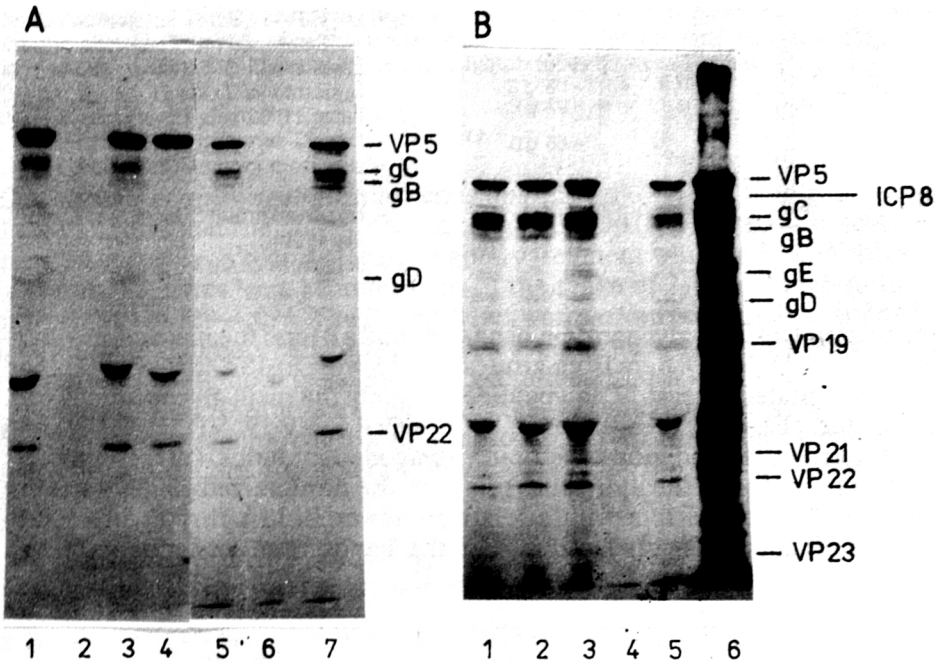


Fig. 3
Precipitation of KOS-infected and control Vero cells with human sera
A - 1, 2 ELISA titre of 2,560 (infected and control cells)
- 3 ELISA titre of 2,560 (infected cells)
- 4 ELISA titre of 1,280 (infected cells)
- 5, 6 ELISA titre of 10,240 (infected and control cells)
- 7 ELISA titre of 10,240 (infected cells)
B - 1, 2, 3, 5 four sera with an ELISA titre of 81,920 (infected cells)
- 4 serum as in lane 3, but control cells
- 6 nonprecipitated infected cells

al., 1991, MoAb No. 170). Group 3 and 4 sera (neutralization titre low but definitely positive) precipitated the main envelope glycoproteins including gD and at least three capsid polypeptides (VP 5, VP 21, and VP 22). The sera of group 6, which showed lower ELISA titres, precipitated less intensive polypeptide bands than those of group 5 (not shown). The group 5 sera reacted with the same viral proteins as did the immune rabbit serum (Fig. 2-B, lane 7).

The relationship between the ELISA titre and the number and intensity of precipitated protein bands is demonstrated on Fig. 3. A batch of sera with ELISA titres from 2,560 to 160,000 (10 of each titre) was used to precipitate the

KOS-infected and noninfected ^{14}C-labelled cells. Most abundant bands were precipitated by sera showing the highest ELISA titre. In addition to the main glycoproteins (gC, gB, gE and gD) and capsid proteins (VP 5, VP 19, 20, 21, 22, 23); further structural as well as some nonstructural virus-coded proteins (ICP8), altogether 16–18 bands reacted with these sera. In contrast, sera with lower titres (2,560 to 5,120) precipitated the gB/gC complex, VP 5, VP 21 and faintly the gD. Intensive precipitation of VP 5 band was probably mediated by a heterologous reaction; it was present at all sera including the ascitic fluid (MoAb), but no serum precipitated this band from the noninfected cell extract.

Discussion

Sera revealing ELISA titres of 20,480 or higher (NA antibody titres in the presence of complement of at least 128 or higher, group 5 in Table 1) reacted with the envelope glycoproteins gB, gC, gE and gD, and with the capsid polypeptides VP 5, VP 19, VP 21, VP 22, VP 23 as well as with at least one nonstructural polypeptide, the ICP8. The precipitation pattern of such human sera resembled to that of immune rabbit serum. The same precipitation pattern was found with mouse serum at late intervals post-infection (McKendall *et al.*, 1988), the number of precipitated polypeptides being different at early and late post-inoculation intervals or after antigenic booster. Human sera tested so far showed similar reactivity, i.e. with the envelope glycoproteins and with the capsid polypeptides (Ashley *et al.*, 1985; Bernstein *et al.*, 1985; Rabie *et al.*, 1991). For designation of viral proteins we used the conventional nomenclature of HSV-1 glycoproteins: gC – 125–9 kD, gB – 115–9 kD (Spear, 1976; Eberle and Courtney, 1980), gD – 59–60 kD (Spear, 1976; Adler Stortz *et al.*, 1983), gE – 75–80 kD (Baucke and Spear, 1979; Ashly *et al.*, 1985). The nomenclature of HSV-1 capsid proteins has been adopted from Honess and Roizman (1973) and from Cohen *et al.* (1980). The high M_r polypeptide VP 1 (ICP 1–2, Bookout and Levey) was not precipitated by our sera, although others found that it may be precipitated by human serum (Rabie *et al.*, 1991).

The sera, which did not neutralize the virus but showed definitely positive low ELISA titres (up to 2,560) precipitated at least three HSV proteins, namely the gB/gC complex and VP 5 (155 kD). It is known that neutralization of the HSV infectivity is predominantly associated with the anti-gD antibody (Eing *et al.*, 1989). Apparent gD bands were precipitated with sera showing satisfactory neutralization titres (groups 4 and 5). Group 6 sera, which showed a low NA titre in the absence of complement and contained predominantly complement requiring neutralizing antibodies (Yoshino *et al.*, 1965; Taniguchi and Yoshino, 1965) reacted more weakly with the glycoproteins, some of them precipitated a very faint gD band only while other did not precipitate this band at all (Fig. 1).

We conclude that, in accordance with Norrild (1981, 1985), human serum reacts with at least two HSV-1 glycoproteins, the intensity of the reaction being dependent on the ELISA titre. The reaction with gD may be related to the NA titre. Sera with low ELISA titres react faintly with the gB/gC complex only. This reaction occurs probably due to the IgG class antibody, as class IgM and IgA antibodies react predominantly with the low M_r capsid polypeptides and with a 80 kD structural protein (Lehtinen *et al.*, 1989). The low grade ELISA, positive non-neutralizing or slightly neutralizing antibodies (sera groups 2 and 3, Table 1) probably persisted from a primoinfection experienced a long time ago, which was not been boostered by secondary reinfection or recurrencies. Our observations, however, bring no support for the notion that precipitation pattern of a given serum would distinguish the primary and recurrent infections.

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