

Age-specific incidence of neutralization antibodies of Herpes simplex virus

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SUMMARY

Sera of 1255 individuals from Novi Sad, varying in age from < 1 month to 69 years, have been tested for neutralization antibodies to Herpes simplex virus type 1.

The eight newborns tested and 97 % of the 507 adults were positive, with titres ranging from 1/4 to 1/256. The titres in newborns were significantly lower than the titres in adults.

After birth the maternal antibodies declined rapidly and 94 % of infants at the age of > 6 months and < 2 years were negative.

After the first year infants in Novi Sad start to acquire herpes-neutralizing antibodies actively, reaching a 50 % incidence of positives between the 2nd and 3rd year of age.

Age-specific incidence rates of herpes positives found in Novi Sad have been compared with those reported from Edinburgh, Freiburg i. Br. and Louisiana. Possible influences of several circumstances upon the incidence rate of positives detected by the neutralization test are discussed.

INTRODUCTION

The results of the first systematic study in Yugoslavia, on neutralization antibodies of Herpes simplex virus, were conducted on a group of 309 individuals of various age groups, coming from the community of Novi Sad (Mašić, 1972). We are now presenting the results of a similar study, conducted on 1255 individuals, which includes also the findings in the group of 309 persons already described. The results discussed here refer thus to serum samples collected during a period of seven years (1968–74).

MATERIALS AND METHODS

Neutralization tests were conducted with a strain of Herpes simplex virus type 1 kindly supplied by Dr Mileva Dimić from the Institute for Immunobiology and Virology, Torlak-Beograd.

Primary cultures of trypsinized kidney cells of young rabbits were grown at 37° C., in growth medium 199 (pH 7.2–7.3, with 20 % calf serum) and in maintenance medium 199 (pH 7.5 with 5 % calf serum). Both media contained penicillin 100 units/ml. and streptomycin 100 µg./ml.

Cell cultures were grown in tubes for about 5 days with one fluid change after 48 hr. On cultures incubated at 35° C. the virus gave an ID 50 mostly in dilutions of 10^{-6.5}.

As early as 24 hr. after inoculation with 10⁻¹ virus, about 50% of the cells showed a pronounced CPE, and in cultures inoculated with 10⁻³ virus signs of an initial CPE were recognizable. Scattered foci of rounded cells first appeared, to be followed by spreading degeneration, and finally all cells fell off the glass. After longer incubation of the cultures infected with higher virus dilutions, there was occasionally formation of syncytial giant cells as well.

Samples of titrated suspensions of the virus, mixed with equal volumes of normal calf serum, were stored for as long as 1 year at -70° C., with a loss in infectivity amounting to *ca.* 1 log unit.

In the period 1968-74 sera of 1255 individuals have been both collected and tested for neutralization antibodies of Herpes simplex virus. They came from the Novi Sad community (about 200,000 inhabitants) and varied in age from < 1 to 69 years. The 1255 specimens consisted of 332 sera collected from apparently healthy persons, and of 923 acute phase sera submitted for examination with various clinical diagnoses. No differences could be detected between the age-specific incidence of positives in these two groups of serum donors.

Sera were stored at -20° C. Thawed samples of sera diluted 1/4 were inactivated for 30 min. at 56.5° C., and diluted in twofold steps from 1/4 to 1/256.

Thawed samples of virus were diluted to contain 100 ID 50 per 0.1 ml.

Each serum dilution was mixed with an equal volume of a virus dilution containing 100 ID 50 per 0.1 ml. After incubation for 1 hr. at 37° C., 0.2 ml. of the serum-virus mixtures were added to tubes showing confluent monolayers, each serum dilution being tested in 2 or 4 tubes. Neutralization titres are expressed as dilutions of sera protecting cells from CPE in 50% of the tubes inoculated. Each serum was titrated once, or repeatedly until clear cut endpoints were obtained.

With the titration of every group of sera, a control of the virus titre has been set up, as well as neutralization tests both with a positive serum of known titre, and with a negative serum sample.

Dilutions of virus and serum were prepared in PBS solution (pH 7.3).

RESULTS AND DISCUSSION

Data presented in Table 1 allow for the following conclusions.

(a) Neutralization titres for herpes virus were detected in nearly all adults and ranged from 1/4 to 1/256. In the group of herpes-positive adults the mean titre was about 1/32.

(b) The eight newborns (age < 1 month) tested were all herpes-positive, but the mean titre amounted just to about 1/13, with individual titres ranging from 1/4 to 1/32.

(c) The incidence of reactors with titres of 1/64 or higher was 36.3% in adults (184/507), and that in newborns was nil (0/8). This finding, as well as the height of the mean titres found in newborns (1/13.5) and in adults (1/33.2) seem to imply

Table 1. Results of neutralization tests

Age	Number of persons with titres							Mean titres*		Incidence of positives (%)	
	<4	4	8	16	32	64	128	256	of all samples		of positives
<1 mo.	—	1	2	3	2	—	—	—	13.45	13.45	8/8 (100)
1- <3 mo.	6	6	2	3	1	1	—	—	5.55	8.90	13/19 (68)
3- <6 mo.	31	10	—	1	—	—	—	—	2.48	4.54	11/42 (26)
6- <12 mo.	61	2	1	1	—	—	—	—	2.16	6.73	4/65 (6)
1- <2 yr.	41	2	—	—	—	—	—	—	2.07	4.00	2/43 (5)
2- <3 yr.	19	1	5	8	1	2	1	—	5.60	16.63	18/37 (49)
3- <7 yr.	61	16	13	28	29	8	2	—	7.32	18.75	96/157 (61)
7- <10 yr.	39	6	6	29	32	11	2	—	10.56	22.44	86/125 (69)
10- <15 yr.	54	18	44	38	53	38	7	—	12.42	20.44	198/252 (79)
15- <70 yr.	16	18	25	96	168	143	40	1	30.38	33.20	491/507 (97)
All	28	80	98	207	286	203	52	1	—	—	927/1255 —

* Geometric means calculated by taking titres < 1/4 as 1/2.

Table 2. A comparison of the incidence (%) of seropositives with type 1 antibodies

Critical titres	Host system	Years of collection	Residence of donors	1/1		1/4		1/8		1/16		Incidence of positives (%)
				Negros	Whites	Prim. rabb. kid. cells	1968-74	BHK 21 cells	1965	Prim. rabb. kid. cells	1968-74	
< 6 mo.	53/57 (93)	30/38 (79)	32/69 (46)	20/23 (87)	20/23 (87)	20/23 (87)	11/69 (16)	1/20	Human fibroblasts
6 mo.- < 3 yr.	67/94 (71)	15/35 (43)	24/145 (17)	17/60 (28)	17/60 (28)	17/60 (28)	13/145 (9)	—	Before 1972
3- < 15 yr.	147/171 (86)	99/117 (85)	380/534 (71)	57/138 (41)	57/138 (41)	57/138 (41)	277/534 (52)	—	Freiburg i.Br.* (Germany)
15-70 yr.	26/27 (96)	47/52 (90)	491/507 (97)	375/489 (77)	375/489 (77)	375/489 (77)	448/507 (88)	—	—

* Together with cases of cervix carcinomas.

that only half the amount of antibodies contained in mothers' sera passes the placenta (see Table 1).

(*d*) In sera of newborns the maternal herpes-neutralizing titres decline rapidly. At the age of 1 year, only 2 of the 43 infants tested were positive, and even those showed a titre of 1/4 only.

(*e*) According to the height of the mean titres shown in seropositive newborns the antibodies seem to decline at a rate suggesting a half-life of 3–11 months.

(*f*) After the 1st year, when the passively acquired antibodies apparently disappeared from the circulation, herpes neutralization titres start to increase, and reach a 50% incidence rate of positives between the 2nd and 3rd year of life. At this age also, the mean titre of seropositives (1/17) amounts just to half of that (1/33) revealed in the group of sero-positive adults.

Table 2 presents a comparison of the incidence of herpes-positives, reported from Louisiana (Buddingh, Schrum, Lanier & Guidry, 1953), Novi Sad, Edinburgh (Smith, Peutherer & MacCallum, 1967) and Freiburg i.Br. (Petersen, Böcker, Fürmaier & Hillemanns, 1972), as determined by the different critical titres adopted.

In the group of donors below the age of 6 months, the incidence of positives in Novi Sad is about 3 times higher (46%) when determined at a critical titre of 1/4, than at a titre of 1/16 (16%). Also in other age-groups similar, and statistically significant, differences were detected between the incidence of positives determined at a critical titre of 1/1 (in Louisiana), and those determined at a critical titre of 1/20 (in Freiburg i.Br.). Moreover, in serum donors of all age-groups from Louisiana, Novi Sad and Freiburg i.Br., the incidence of positives determined according to a lower critical titre was significantly greater than that determined at a higher titre.

These findings are in accord with the well known dependence of the frequency of seropositive reactors upon the height of the critical titres adopted (provided that other circumstances are equal).

The data presented in Fig. 1 and in Table 3 allow for a more detailed comparison of our findings in Novi Sad with those reported from Edinburgh.

Two principal differences are easily discernible:

(*a*) infants, below the age of 2 years, from Edinburgh, show a significantly greater incidence of positives than those from Novi Sad;

(*b*) after the age of 2 years, the incidence of positives becomes greater in residents of Novi Sad than in Edinburgh.

These differences are statistically significant (for raw data see Table 2).

The possibility that such differences could be induced just by the use of a different host system can almost certainly be excluded.

During our work in Novi Sad, the height of critical titres adopted for determination of the incidence of positives, as well as the other circumstances in providing the data referring both to infants below the age of 2 years and to individuals above that age, were all the same. In Edinburgh too, the results referring both to infants below the age of 2 years, and to persons above that age, had been produced under the same conditions. This being so, the display of an inverse incidence

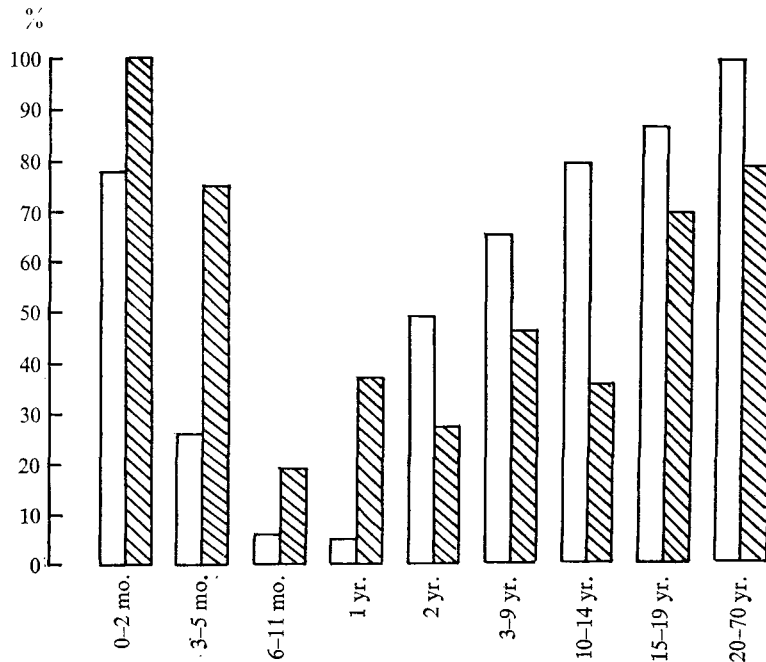


Fig. 1. Percentage of positives in Novi Sad (□) and Edinburgh (▨)

Table 3. *The incidence (%) of seropositives*

Age	In Edinburgh (crit. titre 1/8)		In Novi Sad (crit. titre 1/4)	
	Number	%	Number	%
0- < 3 mo.	11/11	100	21/27	78
3- < 6 mo.	9/12	75	11/42	26
6- < 12 mo.	3/16	19	4/65	6
1- < 2 yr.	8/22	37	2/43	5
2- < 3 yr.	6/22	27	18/37	49
3- < 10 yr.	37/81	46	182/282	65
10- < 15 yr.	20/57	35	198/252	79
15- < 20 yr.	52/75	69	77/90	86
20-70 yr.	323/414	78	414/417	99
All	469/710	—	927/1255	—

of positives among persons below and above the age of 2 years, as revealed in Novi Sad and in Edinburgh respectively, must not be ascribed to any differences between the methods of providing the relevant results, but rather to some peculiarities of the groups of populations compared.

In sera of infants from Edinburgh, the incidence of neutralizing titres declines gradually to a minimum of 19%, reached at the age of 6-11 months. In Novi Sad, the incidence of positives declines to a minimum, as low as 5%, reached in one-year-old children.

Whether the weaker decline of neutralizing titres detected in sera of infants from Edinburgh had been induced by an actually retarded elimination of herpes-

specific maternal antibodies due to the presence of non-specific neutralizing titres, or for some other reason, cannot, for the time being, be soundly substantiated.

In the population of Novi Sad, after the second year of age, the incidence of herpes-positives was significantly higher than that shown by the same age-group in Edinburgh. Sera from Edinburgh were collected in 1965 and the sera from Novi Sad in the period from 1968 to 1974.

As discussed extensively by Smith, Peutherer & MacCallum (1967) as well as by other authors, such differences between the age-specific incidence can be ascribed to specific differences between the two socio-economic environments and to hygienic conditions of the two localities compared. An actual investigation of such circumstances, however, has not been undertaken.

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