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The incidence of complement-fixing antibody to varicella–zoster virus in hospital patients and blood donors

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SUMMARY

The sera of 308 patients, not suffering from varicella or zoster infections, and the sera of 183 blood donors were examined for complement-fixing antibody to varicella-zoster virus. In both groups about 70% of sera from persons aged 11–40 years had antibody titres $\geqslant 1/4$; the incidence was less in the age range 41–60 years and increased in later decades. Antibody titres of 1/16 or 1/32 were noticeably less frequent in those aged 41–60 years than in younger or older groups.

It was concluded that an unchanging titre of 1/16 or 1/32 was of no diagnostic significance. The age distribution of antibody was consistent with the theory that zoster only occurs when antibody has declined.

INTRODUCTION

Although antibody to varicella–zoster virus was demonstrated many years ago (Netter & Urbain, 1926) little is known about the prevalence of antibody in the population.

We have frequently received in the laboratory serum from patients complaining of pain with an apparent nerve root distribution, similar to that of zoster, but with no obvious skin eruption, and if the pain appeared in the chest the provisional diagnosis might be coxsackie virus infection or heart disease. Although no other cause for the pain was found, several of these sera had titres of antibody to varicella–zoster virus of 1/16 to 1/32, but a subsequent rise was rare. It seemed, therefore, that a survey of antibody titres in normal persons and hospital patients would have immediate practical value and might help in elucidating the natural history of varicella–zoster virus infections.

We measured complement-fixing antibody to varicella–zoster virus in all sera sent to the virology laboratory between January and August 1967, and in the sera of 183 blood donors.

MATERIALS AND METHODS

Sera

The routine sera were specimens sent for the diagnosis of common viral infections. Sera from blood donors were kindly supplied by Dr A. E. Preston of the Regional Blood Transfusion Service, Churchill Hospital, Oxford.

On receipt sera were inactivated at 56° C. for 30 min., stored at −20° C., and, if the storage was prolonged, again inactivated before test.

Varicella–zoster antigen

The method of preparing antigen was essentially that of Schmidt, Lennette, Shon & Shinomoto (1964). Human embryo fibroblasts were grown in 6 oz. medical flats, heavily infected with virus and incubated at 36° C. until most of the cells were rounded—usually 4 days. The medium was then discarded, the cells were scraped off with a rubber-tipped glass rod, suspended in 1 ml. veronal buffer for each bottle harvested, frozen, thawed, and disrupted by ultrasonic energy for 3 min. in an H 60 Ultrasonic Cleaner (Headland Engineering Developments Ltd.). After centrifugation at 3000 rev./min. the cell debris was discarded and the fluid stored at −70° C.

Herpes simplex virus antigen

This was prepared by infecting RK 13 cells with a strain of virus isolated from herpes labialis lesions, then harvesting and disrupting as for the V–Z antigen. It was standardized by titration against a serum supplied by the Standards Laboratory, Colindale.

Complement fixation

Tests were done in W.H.O. Perspex trays using 2·5 haemolytic doses of complement and allowing 18 hr. at 4° C. for fixation. The dose of antigen used was determined for each batch by chessboard titration. (See Results.)

RESULTS

Antigen titration

The results of a typical titration of V–Z antigen, as given in Table 1, showed a broad optimum for the dose of antigen reacting with a zoster serum, but no optimum with a varicella serum. For this survey the dose of antigen used was twice the minimum amount giving full fixation at the maximum serum dilution, i.e. a 1/16 dilution of the batch of antigen used in the titration of Table 1.

Specificity of complement fixation

Heterologous antibody rises in varicella–zoster and herpes simplex virus infections have been reported (Kapsenberg, 1964; Ross, Subak Sharpe & Ferry, 1965; Svedmyr, 1965; Schmidt, Lennette & Magoffin, 1969) but Schmidt *et al.* express the opinion that a previous antigenic stimulus with V–Z virus is essential before a herpes simplex infection will elicit a rise in antibody to varicella–zoster virus. There is, at present, no certain way of ensuring the specificity of single determinations, but some check was provided by assembling the results of testing 184 sera for the presence of complement-fixing antibody to both V–Z virus and herpes simplex virus (Table 2). As expected, many sera reacted with both antigens, but 22% reacted only with herpes simplex antigen and 21% reacted only with V–Z antigen, showing that different antibodies were detected by the two antigens.

Table 1. *Chess-board titration of varicella–zoster antigen*

Serum dilution	Degree of complement fixation* with Varicella serum. Antigen dilution 4	8	16	32	Zoster serum. Antigen dilution 4	8	16	32	64	128
8	4	4	3	1	—	—	—	—	—	—
16	4	4	4	2	4	4	4	4	3	1
32	4	4	4	2	4	4	4	4	4	2
64	4	4	4	1	4	4	4	4	4	2
128	4	4	4	1	4	4	4	4	3	1
256	4	4	3	1	4	4	4	4	3	0
512	4	4	3	0	4	4	4	4	3	0
1024	4	4	2	0	3	4	4	4	1	—
2048	4	2	1	0	0	1	1	1	0	—
Control	0	0	0	0	0	0	0	0	0	—

* 4 = Complete fixation; 0 = complete haemolysis; — = not tested.

Table 2. *Sera tested for antibody to V–Z virus and Herpes simplex virus*

		Antibody to H.S.V. Absent	Present
Antibody to V–Z virus	Absent	31	41
	Present	39	73

Total: 184

Table 3. *Titres of antibody to varicella–zoster virus in sera from* 308 *patients*

Age group	No. of patients	Number of sera with titres equal to or greater than 1/4	1/8	1/16	1/32
0–10	19	9 (47)	6 (32)	5 (26)	5 (26)
11–20	59	43 (73)	32 (54)	26 (44)	20 (34)
21–30	98	69 (70)	57 (58)	47 (48)	27 (28)
31–40	35	25 (71)	19 (54)	14 (40)	6 (17)
41–50	23	18 (78)	9 (39)	9 (39)	4 (17)
51–60	26	18 (69)	12 (46)	11 (42)	5 (19)
61–70	19	11 (58)	10 (53)	9 (47)	4 (21)
71–80	20	12 (60)	11 (55)	8 (40)	6 (30)
81–90	9	6 (67)	6 (67)	5 (56)	4 (44)

Figures in parentheses indicate percentages of the total number in each age group.

Antibody in the sera of patients

When sera from patients suspected of having varicella or zoster had been excluded, 308 results remained and these, classified by age group and titre, are shown in Table 3. The lowest percentage of detectable antibody was in the age group 0–10 yr., the period when first infections are being acquired; from 11 to 50 yr., over 70 % of patients had antibody and in the older groups there was a

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slight fall in the incidence. The percentages with titres equal to or greater than 1/8, or 1/16, were not particularly remarkable but did show a similar trend to those with titres of 1/4. The incidence of titres of 1/32, or higher, showed a broad minimum in the group 31–60 yr., with higher percentages in younger and older groups.

Antibody in the sera of blood donors

The results on the sera of 183 blood donors are set out in Table 4 and they show an unequivocal fall in the percentage with antibody, at each of the four levels in the group 41–60 yr. As there were only five donors over 60 yr. the increased percentage in the last group has little significance.

Table 4. *Titres of antibody to varicella–zoster virus in sera from* 183 *blood donors*

Age group	No. of donors	Number of sera with titres equal to or greater than 1/4	1/8	1/16	1/32
11–20	17	12 (70)	10 (59)	8 (47)	1 (6)
21–30	64	39 (61)	24 (37)	14 (22)	4 (6)
31–40	37	26 (70)	15 (41)	7 (19)	0
41–50	33	17 (52)	9 (27)	2 (6)	1 (3)
51–60	27	12 (44)	6 (22)	3 (11)	1 (4)
61–70	5	4 (80)	4 (80)	1 (20)	0

Figures in parentheses indicate percentages of the total number in each age group.

DISCUSSION

The results from the two sets of sera differ in that the patients showed higher percentages with antibody than did the blood donors, presumed to be healthy, and no satisfactory explanation for this observation can be offered. On the other hand, the two series concur in showing a fall in the proportion of individuals with antibody in the group 41–60 yr. Figures presented by Schmidt *et al.* (1969), in a study of the immunological relationship between herpes simplex and varicella–zoster viruses, provide information on 75 sera from San Francisco which show a similar trend, though they do not comment on it. They give, by age group, the number of patients with herpes simplex virus infections who had complement-fixing antibody to V–Z virus in the acute-phase serum: 1–10 yr., 6%; 11–20 yr., 59%; 21–30 yr., 75%; and 31 yr. and over, 31%.

All these results contrast sharply with the reported incidence of antibody to herpes simplex virus as illustrated by a recent survey by Smith, Peutherer & MacCallum (1967), who also give references to 18 similar surveys, all of which show that the percentage of individuals with antibody to herpes simplex virus increases steadily with increasing age.

It is now generally accepted that herpes–zoster is a localized resurgence of virus which has remained latent from a previous attack of varicella and Hope-Simpson (1965) suggested that this reactivation can only occur when antibody has fallen

below some critical level. As varicella usually occurs in the first 20 years of life and zoster mostly in those over 50 yr. old, the Hope-Simpson theory postulates a decrease in antibody level with advancing years and, as a corollary, one might expect in the higher age groups a lower percentage of the population to have detectable antibody. This, in fact, is what we have found.

In his review of 192 cases of herpes zoster, seen in general practice, Hope-Simpson records the incidence per 1000 population, per year, as 2·58–2·92 between the ages of 20 and 49 years, rising to 5·09 in the sixth decade and progressing to 10·1 in the ninth decade. The incidence of zoster began to increase in the age groups in which we found the minimum incidence of antibody.

Whether or not the onset of an attack of zoster is determined by the pre-existing antibody titre is difficult to decide, because by the time the first serum is collected a secondary rise of antibody may have begun. There are reports in the literature of zoster patients who did not have detectable antibody in the acute-phase serum and in our records of 74 cases of zoster (of which 71 had convalescent titres $\geqslant 1/64$) 15 had a titre of less than 1/4 in the first serum.

Although this type of complement fixation test is satisfactory for demonstrating changes in antibody titre in sequential samples of serum, it is not a good technique for single determinations. Neutralization tests with V–Z virus would be very laborious to apply to large numbers of sera (Caunt & Shaw, 1969) and the immunofluorescent technique (Schmidt, Lennette, Woodie & Ho, 1965), which we used on 60 sera, gives no more precision than the complement fixation test and is more time-consuming. However, when applied to many sera, a total of 491, it is unlikely that the imprecision of a single CF test has obscured the pattern of incidence.

We conclude that, with the technique used, unchanging titres of 1/16 or 1/32 have no diagnostic significance. Further, the age distribution of antibody to V–Z virus is consistent with the theory that latent virus only becomes active when antibody has declined.

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