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Decrease of the Lymphoproliferative Response to Varicella-Zoster Virus Antigen in the Aged

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Humoral antibodies and specific cellular immune reactions (proliferative immune response in the lymphocyte transformation test) to varicella-zoster virus antigen were measured in children, young adults, and elderly people. In children and young adults, the humoral varicella-zoster-specific antibodies and the virus-specific cellular immune response generally coincided. In the over-60 age group, however, a discrepancy was often observed between these parameters. Ninety percent of the elderly subjects showed humoral antibodies, but only 64% still had a measurable varicella-zoster-specific immune response. There was no correlation between the magnitude of the virus antigen-specific immune response and the mitogen-induced lymphoproliferative response (phytohemagglutinin stimulation). One in three elderly people, therefore, showed no cellular immune response to the varicella-zoster virus antigen, and this person could probably be regarded as a potential herpes zoster patient.

The occurrence of herpes zoster despite the persistence of humoral antibodies suggests that specific cell-mediated immune responses may play a vital role in protection against the reactivation of latent varicella-zoster (VZ) virus (12). Herpes zoster is a disease of the second half of life. In persons over 50, the morbidity rate is 5/1,000 per year (11). Herpes zoster generally appears some decades after the initial infection with VZ virus; the age distribution of varicella virus is that of a typical disease of childhood, 93% of the population having had the disease by the age of 15 (9).

Cellular immunity can be determined on the basis of a number of different parameters which are functions or products of either immune or sensitized cells or of the interaction of immune cells with a specific antigen (5). In a number of instances, during the course of a virus infection or after immunization with a viral vaccine, an antigen-specific cell-mediated immune response has been measured by the lymphocyte transformation test (2-4, 6-8, 15). Depending upon the sensitivity of the method, either a merely transient or a lasting lymphoproliferative reaction has been determined.

Using this test, Hayes and Feldman (7) determined cellular immunity to VZ virus and found that it was clearly age dependent. Although there was appreciable individual variation, adults (age not stated) on average showed an antigen-specific lymphocyte stimulation several times greater than that of children, although the initial infection with VZ virus had taken place a considerable time previously. However, Hayes and Feldman did not investigate whether the increased capacity for antigen-specific stimulation found in adults was retained or diminished in old age.

This study was carried out to compare the antigen-specific immune response in children, young adults, and elderly people in order to establish whether there is a correlation between the frequency of a diminished cellular immune response and the statistically established increase in the incidence of herpes zoster in old age.

MATERIALS AND METHODS

Study population. Three age groups of healthy subjects were studied. Group I consisted of 52 randomly selected healthy children aged 4 to 15 years. Group 2 consisted of adults aged 20 to 40 years. Forty-three randomly selected medical students and staff from the hospital were pretested for humoral antibody against VZ virus. From another group of more than 400 medical students, those who gave a negative or unknown history of VZ infection were tested for antibody. Eleven subjects with no humoral antibody were included in age group 2. Group 3 consisted of 110 randomly selected persons aged over 60 years who had been found to be in good clinical condition.

Laboratory tests. (i) Unspecific stimulation of lymphocytes with mitogen. The stimulation of lymphocytes with phytohemagglutinin (PHA) was carried out essentially by the method of Pellegrino et al. (13). Briefly, heparinized whole blood was diluted 1:20 with RPMI 1640 culture medium containing glutamine and supplemented with 10% fetal calf serum, penicillin (100 U/ml), and streptomycin (100 μg/ml). Ten 0.1-ml sam-

ples were placed on cell culture microtiter plates, and 0.5 μg of PHA-P (Seromed; Munich, West Germany) per 0.1 ml was added to supplemented RPMI 1640 medium and to unsupplemented control culture medium. The plates were covered and incubated in a 5% CO_2 incubator at 37°C for 5 days. ^{125}I-labeled I-5-iodo-2'-deoxyuridine plus 10^{-6} M 5-fluoro-2'-deoxyuridine (0.25 μCi/20 μl) was added. After incubating for a further 18 h, the cells were harvested on paper disks with a Skatron cell harvester, and each sample was counted for 1 min with an LKB 1270 gamma counter. The geometric mean of five values was calculated.

(ii) Specific stimulation of lymphocytes with VZ antigen. The lymphocyte stimulation test was performed as described by Hayes and Feldman (7) and by Ruckdeschel and Mardiney (15) with minor modifications.

For VZ antigen preparation, MA184 (Microbiological Associates, Bethesda, Md.) foreskin fibroblast cultures were infected with the Webster strain of VZ virus (2×10^5 plaque-forming units per Roux bottle). After incubation for 4 days at 35°C in minimal essential medium plus 2% fetal calf serum, the cultures were treated for 1 min with 0.1% ethylenediaminetetraacetic acid in phosphate-buffered saline. The cells were centrifuged at 600 × *g* for 30 min, washed three times with phosphate-buffered saline, and resuspended in 5 ml of buffer. After sonication (30 to 40 s), the disrupted cell preparation was stored at −70°C. Control antigen was prepared from uninfected cells as described above.

In the test procedure, 10 ml of whole blood was collected in a syringe containing heparin (50 U/ml) which was then fixed in an upright position on the inner wall of an incubator. The erythrocytes were allowed to sediment for 90 min at 37°C. The leukocyte-rich plasma was recovered and centrifuged for 10 min at 600 × *g*; 1.5 ml of leukocyte-free plasma was saved for the determination of humoral antibody and for addition to the culture medium for the lymphocytes. The leukocyte pellet was suspended in the remaining plasma, and the total number of leukocytes and lymphocytes was determined.

Triplicate cultures, each containing 5×10^5 lymphocytes per ml of RPMI 1640 medium suplemented with penicillin, streptomycin, and 10% autologous plasma, were placed on a microtiter plate. The 1-ml amount required for one determination was distributed equally in five wells arranged in such a way that they could be harvested on one filter paper. VZ antigen and control antigen were diluted in RPMI medium without plasma, and 20 μl was added to each well to give final dilutions of 1:100 and 1:200. After preliminary tests, the control antigen was used only at a final dilution of 1:100. The plates were covered and incubated for 6 days in a 5% CO_2 incubator at 37°C. The addition of tracer, harvesting, and counting were done in the same way as described for the cultures stimulated by PHA.

The higher of the two geometric mean values of the VZ antigen-stimulated cultures was regarded as the optimum dose response and taken into account for the evaluation of the results. Two different indices were calculated: the stimulation index was defined as counts per minute of VZ antigen/counts per minute of control antigen; the specific increase was determined as counts per minute of VZ antigen − counts per minute of control antigen. A stimulation index below 2 or a specific increase below 1,000 cpm was interpreted as an absence of a specific cellular immune response to VZ antigen. A low-to-medium response was considered to take place with stimulation values of 2 to 5 or a specific increase of 1,000 to 5,000 cpm. Higher values were regarded as a strong cellular immune response to VZ antigen.

In PHA stimulation, the normal values for the specific increase ranged from 2×10^4 to 6×10^4 cpm, and the stimulation indices ranged from 50 to 400. The capacity for mitogen-induced lymphocyte transformation was considered to be reduced at values below 5,000 cpm and a stimulation index below 20.

(iii) FAMA test. Humoral antibody determinations were performed with the test for VZ fluorescent antibody to membrane antigen (FAMA) as described by Williams et al. (17), using glutaraldehyde-fixed VZ-infected foreskin fibroblasts (strain MA184) (18).

RESULTS

Nonspecific stimulation of lymphocytes with mitogen. In age groups 1 and 2, all subjects responded normally to PHA in the lymphocyte transformation test (32,900 ± 12,700 cpm). In age group 3, the average specific increase was 26,600 ± 18,000 cpm. Four of 110 persons had a stimulation index below 10; 5 had an index between 10 and 20, corresponding to a low nonspecific cellular immune response.

Specific cellular immune response to VZ antigen. Table 1 presents the distribution of negative, low, and high responders to the in vitro lymphocyte transformation assay with VZ antigen. Each age group contained several subjects with or without measurable humoral antibody (FAMA+ and FAMA−).

(i) Age group 1 (4 to 15 years old). In most

TABLE 1. *VZ antigen-dependent lymphocyte transformation in different age groups with or without humoral antibody to VZ virus*

Subjects	No. with stimulation index of:							
	0–1	1–2	2–3	3–5	5–10	10–20	20–50	50
FAMA+								
Group 1	0[a]	5	5	9	4	4	2	1
(*n* = 30)	5 (17)		14 (47)			11 (36)		
Group 2	0	0	3	4	7	14	11	4
(*n* = 43)	0		7 (16)			36 (84)		
Group 3	6	27	12	24	12	13	5	1
(*n* = 100)	33 (33)		36 (36)			31 (31)		
FAMA−								
Group 1	11	9	0	0	1	0	1	0
(*n* = 22)	20 (91)		0			2 (9)		
Group 2	3	2	3	0	1	1	1	0
(*n* = 11)	5 (46)		3 (27)			3 (27)		
Group 3	2	3	1	3	1	0	0	0
(*n* = 10)	5 (50)		4 (40)			1 (10)		

[a] Numbers in parentheses are percentages for two groups.

cases, the cellular and the humoral responses coincided with regard to presence or absence, but in roughly one of five FAMA+ children, only the humoral antibody could be measured. On the other hand, 1 of 10 FAMA− subjects had a well-developed cellular reactivity but no detectable humoral antibody. This confirms the observation of Hayes and Feldman (7), who found a divergence between the humoral and the cellular responses in 1 of 10 FAMA+ and 1 of 16 FAMA− children.

(ii) Age group 2 (20 to 40 years old). In all FAMA+ subjects, the cellular immune response coincided with the presence of humoral antibody, and the stimulation index was frequently high. Of 11 FAMA− persons, selected from a large group of medical students with a negative or unknown VZ history, 6 show a remarkable cellular immune response. They must have had a previous varicella infection.

(iii) Age group 3 (over 60 years old). In this group, the divergence between measurable humoral and cellular immunity became a frequent occurrence. One of three FAMA+ subjects showed no cellular immune response, and one in two FAMA− subjects still retained cellular reactivity but no humoral antibody production. One of 11 had lost both functions, and it was very unlikely that he had never encountered a varicella infection during his long life.

Stimulation index and specific increase. During a culture period of 6 days, the unstimulated lymphocytes in the control cultures did not remain inactive and showed a certain degree of spontaneous transformation reflecting the immune processes going on in each individual. Therefore, tracer incorporation in the control culture occasionally increased from a normal value of around 1,000 cpm to values of 4,000 to 6,000 cpm without any irregularities in the culture conditions. Since high values of tracer incorporation in the control culture affected the stimulation index, the specific increases of the different age groups are presented (Table 2). In age group 3, the tracer incorporation values showed a notable decrease.

Correlation between nonspecific and antigen-specific cellular immune response. A connection probably exists between a low antigen-specific lymphocyte response and a depressed nonspecific response to a mitogen such as PHA. Since a low nonspecific cellular response could be found in only a few subjects of age group 3, and since in this age group a low antigen-specific cellular immune response was frequent, the stimulation indices of PHA and VZ antigen in 100 FAMA+ subjects over 60 years old were compared (Table 3). No correlation was found between the two parameters.

TABLE 2. *Specific increase in tracer incorporation in VZ antigen-dependent lymphocyte transformation*

Subjects	No. with specific increase of:		
	<1,000	1,000–5,000	>5,000
FAMA+			
Group 1 (n = 30)	4 (13)[a]	15 (50)	11 (37)
Group 3 (n = 43)	0	10 (23)	33 (77)
Group 3 (n = 100)	36 (36)	44 (44)	20 (20)
FAMA−			
Group 1 (n = 22)	20 (91)	0	2 (9)
Group 2 (n = 11)	8 (73)	2 (18)	1 (9)
Group 3 (n = 10)	6 (60)	4 (40)	0

[a] Numbers in parentheses are percentages.

TABLE 3. *Magnitude of nonspecific cellular immune response compared with antigen-specific immune response in 100 FAMA+ subjects of group 3*

Stimulation index, VZ antigen	Stimulation index, PHA				
	1–10	10–20	20–50	50–100	>100
C–2 (n = 33)	2[a]	2	9	14	6
2–5 (n = 36)	2	2	7	12	13
>5 (n = 31)	0	1	5	12	13

[a] Number of subjects belonging to each group.

DISCUSSION

In the study of adults aged 20 to 40 years, all subjects with humoral varicella antibodies were also found to have specific cell-mediated immune responses to varicella antigen. In this age group it was difficult to find individuals who were negative to varicella in the FAMA test; only 11 of more than 400 medical students examined were found to have no humoral varicella antibodies. Six of these subjects had a positive cellular immune response to varicella antigen even though there was no evidence of humoral varicella antibodies. These subjects must have had a varicella infection at some time. Varicella is rare in adults; according to Preblud and D'Angelo (14), only 2.5 varicella infections occur per 100,000 adults each year. In the children studied, humoral and cellular varicella immune reactions also generally occurred concomitantly.

To explain the differing results among individuals, a number of hypotheses may be proposed. Determination errors in the testing of humoral varicella antibodies are virtually ruled out. All FAMA tests were repeated several times, and the results showed only small fluctuations in the antibody titers. Errors in the determination of

specific cellular immune responses cannot be excluded in all cases. Since these cellular tests could be carried out only with freshly taken blood samples, repetition was usually impossible.

However, multiple determinations in hospital personnel showed that certain fluctuations were possible; a change from positive to negative or from negative to positive results did not occur. We consider it most probable that in the children with differing results the persisting cellular or humoral immune response was below the sensitivity limit of the test method. Hayes and Feldman (7) state that immune reactions to VZ viruses are appreciably smaller in children than in adults. Since the children in our study were constantly coming into contact with varicella viruses, the specific immune reactions were boosted to such a degree that humoral and cellular immune reactions would persist concomitantly into adulthood in almost all cases.

Humoral antibody to VZ viruses could be detected in almost all subjects over 60, but cellular immune responses could be detected only in approximately two-thirds of these subjects. All of the test subjects were clinically healthy and were not under the influence of cytostatics. The cellular immune response to an unspecific mitogen (PHA) was found to be in the same range as in the group of relatively young adults in more than 90% of the cases.

It is known that the cellular immune response to PHA and pokeweed mitogen diminishes in old age (16), although in our study only 8% of the subjects over 60 had a reduced capacity for mitogen-induced lymphocyte transformation. It is not clear whether this diminution was due to a reduction in the functional capacity of the T-lymphocytes or to soluble factors (10). On the other hand (as, for example, in the case of influenza immunization), it is known that old people are also capable of a humoral immune response (1). We do not know whether the diminution in old age of the cellular immune response to VZ antigen is specific to this virus or if it also applies to the capacity for stimulation by other virus antigens.

The diminution of the specific cellular immune response to VZ antigen parallels the increasing morbidity from herpes zoster infection with increasing age. One is justified in assuming that subjects with a negative cellular immune reaction to VZ antigen are potential herpes zoster patients. However, this does not tell us what factors trigger the development of clinical herpes zoster in individual cases.

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