Immune Responses to Varicella-Zoster in the Aged

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• Skin test reactivity and in vitro lymphocyte stimulation responses to varicella-zoster (VZ) were examined in a large normal population ranging in age from 6 months to 93 years. Waning of cellular immunity, as examined by skin delayed hypersensitivity, began at age 40 years. Skin test responses to phytohemagglutinin, however, remained positive into the eighth decade of life. In vitro lymphocyte stimulation responses to VZ were usually positive (stimulation index $\geq$ 2.5) until age 60 years, after which time levels, as observed with nonimmune individuals, were often demonstrated. Antibody levels, as measured by fluorescent antibody to membrane antigen, remained positive into the ninth and tenth decades of life. This was especially so with a history of reactivation (zoster) VZ infections, while skin test and in vitro responses were rarely positive in those individuals. Thus cellular, as contrasted with humoral, immunity decreases with advancing age, which may account for a propensity to reactivation of VZ virus.

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Primary varicella-zoster (VZ) is a relatively benign viral disease recognized clinically as chickenpox, which infects essentially everyone in this country. The virus becomes latent, probably in all hosts, to be reactivated at a later time for reasons as yet poorly understood. The clinical presentation of reactivation, termed "zoster" or "shingles," is experienced by an estimated 0.2% to 2% of the population, most of whom are elderly adults[1,2] or immunosuppressed individuals[3] who have had chickenpox as children. Immunosuppression may result from underlying malignant neoplasms such as Hodgkin's disease,[4] immunosuppressive therapy, or both. Depressed cellular immunity has been documented in such patients. Less well defined is immune reactivity to specific antigens such as VZ in the elderly. It is apparent that a better understanding of changes in immune capabilities as a person ages might offer insight into control of VZ infection with application to other groups such as cancer patients. The present studies were, therefore, designed to investigate both cellular and humoral immune response to VZ in age groups from infancy to very elderly.

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MATERIALS AND METHODS

Study Population

Volunteers were selected from employees and outpatients at the University Hospital Little Rock, Ark, and patients followed up by the Geriatrics Service at the Little Rock Veterans Administration Medical Center. Particular emphasis was placed on including only individuals who were free from an acute or chronic disease that might affect immunological competence and those with optimal nutritional status. Institution-approved informed consent was obtained from each participant so that blood could be drawn and skin tests applied. Each participant completed all aspects of testing described below whether or not he was considered immune to VZ.

Immunological Assays

The following in vivo and in vitro correlates of immune function were examined: (1) skin testing with VZ antigen and phytohemagglutinin (PHA), (2) mitogen (PHA)- and antigen (VZ)-induced lymphocyte stimulation, and (3) antibody to VZ.

Skin Testing

Varicella-zoster virus antigen was prepared from the Scott strain of VZ and human diploid cells. Details of its production and evaluation were described previously.[5] Phytohemagglutinin was purified from a commercial lot of reagent with a total dose of 20 μg employed for skin testing. A volume of 0.1 mL of VZ antigen, PHA, or control (normal saline) material was injected intradermally into the forearm, and reactivity was measured at 48 and 72 hours. The maximum response was recorded as millimeters of erythema or induration. A reaction was considered positive if induration or erythema was 5 mm or more.

Mitogen- and Antigen-Induced Lymphocyte Stimulation

Lymphocytes were separated from peripheral whole blood by centrifugation on a Hypaque-Ficoll gradient as previously described.[6] Using a biopipette, 0.1 mL of the lymphocyte suspension (2×10^5 lymphocytes) was added to an equal volume of various concentrations of PHA and VZ antigen. A harvesting apparatus, previously described and technically refined for our use, was employed for separation of the stimulated lymphocytes on glass-fiber filters, for washing of these cells, and for recovery of tritiated thymidine uptake.[7] The PHA cultures were incubated for three days using a five-hour tritiated thymidine pulse and VZ cultures for five days with a 24-hour pulse. Results are expressed as a stimulation index: counts per minute of tritiated thymidine uptake for lymphocytes incubated with PHA or VZ, divided by counts per minute after incubation with medium alone.

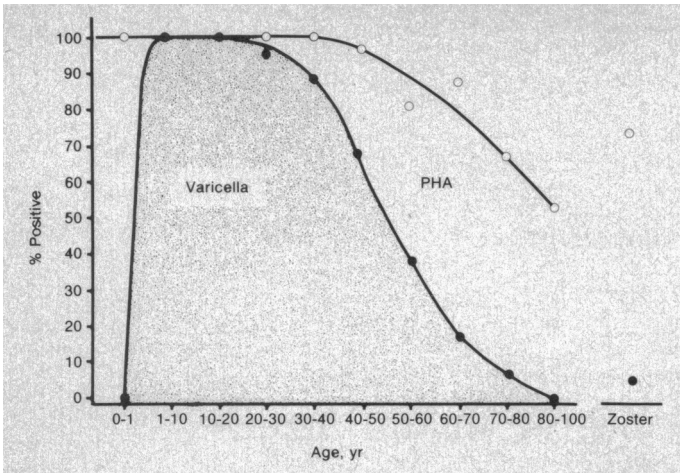

Fig 1.—Percent positive skin test responses to varicella-zoster (closed circles) and phytohemagglutinin (PHA) (open circles) according to age. Skin reactivity considered positive if induration or erythema was 5 mm or more at 48 or 72 hours.

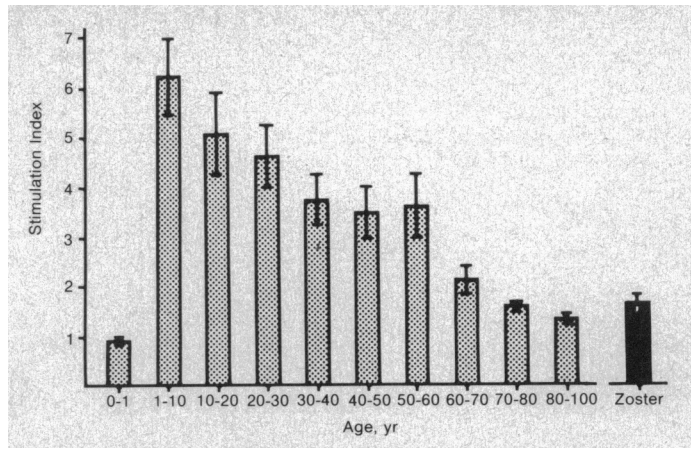

Fig 2.—In vitro varicella-zoster (VZ)–induced lymphocyte stimulation according to age and expressed as stimulation index: counts per minute of tritiated thymidine uptake for lymphocytes incubated with VZ, divided by uptake after incubation with medium alone.

Table 1.—Varicella-Zoster Skin test Reactivity

Age, yr	No. Tested	Varicella-Zoster No. Positive	Varicella-Zoster % Positive	Phytohemagglutinin No. Positive	Phytohemagglutinin % Positive
0-12 mo (nonimmune)	13	0	0	13	100
1-10	24	24	100	24	100
10-20	9	9	100	9	100
20-30	24	23	96	24	100
30-40	27	24	89	27	100
40-50	26	18	69	25	96
50-60	31	12	39	25	81
60-70	17	3	18	15	88
70-80	12	1	8	8	67
80-100	13	0	0	7	54
Zoster (60-100)	14	1	7	10	71

Table 2.—Varicella-Zoster In Vitro Lymphocyte Stimulation

Age, yr	No. Tested	Mean ± SEM*
0-12 mo (nonimmune)	13	0.99 ± 0.05
1-10	24	6.28 ± 0.79
10-20	9	5.01 ± 0.85
20-30	19	4.62 ± 0.64
30-40	17	3.75 ± 0.51
40-50	18	3.52 ± 0.51
50-60	20	3.60 ± 0.69
60-70	12	2.10 ± 0.26
70-80	12	1.56 ± 0.13
90-100	13	1.39 ± 0.10
Zoster (60-100)	14	1.63 ± 0.19

*Expressed as a stimulation index: counts per minute of tritiated thymidine uptake for lymphocytes incubated with varicella-zoster antigen, divided by uptake after incubation with medium alone.

Varicella-Zoster Antibody

Serum antibody to VZ was measured by fluorescent antibody to membrane antigen.[8] Briefly, unfixed tissue culture cells were incubated with various dilutions of test serum, washed, incubated with fluorescein-labeled antihuman IgG, and examined by fluorescence microscopy. Surface fluorescence at serum dilutions of 1:8 or more was considered positive.

RESULTS

Skin test reactivity for various age groups is summarized in Fig 1. No patient demonstrated changes following injection of normal saline (control negative material). After age 40 years, the incidence of positive skin test responses to VZ progressively decreased, while PHA responses showed consistent delayed hypersensitivity reactions into the eighth decade of life. Therefore, as judged by these in vivo assays, immunity to VZ wanes beginning at 40 years of age, while more nonspecific responses may persist in later years.

In vitro lymphocyte stimulation with PHA supported previously reported data demonstrating declining reactivity with age. Results for subjects in their eighth decade of life or older had mean counts per minute approximately half those from volunteers younger than 40 years. Lymphocyte proliferation responses, specific for VZ antigen, are tabulated in Fig 2. Mean values were above control positive determinations (stimulation index > 2.5) until age 60 years. This suggests adequate immunity for most individuals until quite late in life. Results are similar for measurement of VZ antibody. Most "normal" individuals up to age 80 years had protective titers (Table 1).

Elderly patients (60 to 100 years) with a history of reactivation disease, ie, zoster, usually had measurable antibody but negative skin test and stimulation responses to VZ antigen (Fig 1 and 2 and Tables 1 and 2).

COMMENT

Aspects of cellular immune function in the elderly have been the subject of previous investigations, with most suggesting a waning with age. Decreased lymphocyte stimulation, using either PHA or concanavalin A, has been documented in a number of reports,[9-11] as well as in the present one. However, interpretation of these data is difficult since mitogen stimulation is a rather gross measurement of immune capability. More recently, similar results were found using VZ specific lymphoblastoid proliferation assays.[12] The latter study included 14 elderly adults (over 60 years of age), who were compared with 15 young

subjects (under 35 years of age). This report also demonstrated equivalent proliferative responses to other antigens (streptokinase-streptodornase and PPD), antibody titers to VZ, and interferon production after stimulation with VZ.

The present studies attempted to more clearly define the age at which specific cellular immune reactivity begins to diminish. Skin testing was included since this test would be readily applicable in the general clinical setting. It is not presently apparent whether more complex in vitro assays would actually offer any advantage over the skin-testing approach in evaluating cell-mediated immunity to VZ. With this in vivo screen, waning of immunity was recognized to begin at about age 40 years (Fig 1). By age 60 years, most individuals had negative skin test responses. In vitro measurement, on the other hand, suggests that cellular immunity persists in most individuals until age 60 years (Fig 2), after which time reactivity decreases. This follows the clinical correlate, ie, propensity to reactivation disease, more closely than skin test results.

Even after age 80 years, 54% of volunteers manifest reactions to PHA. Many reactions were only 5 to 10 mm of erythema without induration, but these would still be considered positive. Phytohemagglutinin, as a skin test reagent, has been shown to produce more consistently positive responses than antigen preparations.[13] More recently, however, investigations have suggested that some skin test responses may not be indicative of delayed hypersensitivity since patients with proven cellular immune deficiency occasionally manifest erythema or induration following injection.[14] Thus, the PHA skin test response may represent a more nonspecific immunological event. With few exceptions, the only young patients we have encountered with negative PHA skin test responses were those with severe primary or secondary immune deficiency. This has also been the experience for others in studies of cancer patients.[15] However, a more comprehensive evaluation of other skin test antigens in the elderly would better delineate whether decreased cellular immunity is a selective one for VZ since PHA does not discriminate subtle defects.

In contrast to the decline in cellular immune responses to VZ with aging, humoral immunity remains relatively intact. This, once again, refutes the Hope-Simpson hypothesis[16] that reactivation VZ disease is a consequence of antibody titers falling below critical levels. Most published data have also confirmed the presence of antibody both in the elderly and in patients with zoster.[17]

Fourteen elderly volunteers (60 to 100 years) had a history of shingles, with eight reporting two or more episodes. The course of this disease tended to be protracted, frequently persisting for more than a month. The most difficult management problem was the pain that accompanied the vesiculation. None of the 14 patients, however, were treated with antiviral agents. The VZ immune function testing in these subjects revealed consistently high antibody titers, but essentially absent cellular immune reactivity. Thus, waning of this aspect of host defense probably accounts for a propensity to reactivation disease and development of zoster does not appear then to stimulate conversion of VZ cell-mediated responses. The individual, therefore, remains predisposed to recurrent episodes.

The application of these observations to cancer patients is quite relevant. These patients represent the group at highest risk to VZ disease and the ones most likely to require medical intervention for progressive disease. Depression of cellular immunity is also considered the most important host factor for predisposition to disease in these individuals. Treatment trials, such as antiviral chemotherapeutic agents, are being examined. Preliminary results indicate that some aspects of immunotherapy in conjunction with antiviral agents may provide the clinical armamentarium so long awaited for combating this ubiquitous viral agent.[18] An understanding of the immunology of VZ virus disease is, of course, essential for guiding efforts at therapy. Combining the present data and our experience with the same assays in cancer patients,[18] it appears that the immunological status of the aged and the cancer patient is quite similar and that waning of cellular immunity to VZ in both groups is related to subsequent viral reactivation.

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