References


Interferon Response to Mitogens and Viral Antigens in Elderly and Young Adult Subjects

Herpes zoster, a disease thought to be due to reactivation of varicella-zoster virus (VZV), is more common in elderly individuals than in the rest of the population. Hope-Simpson [1] reported the disease to be relatively uncommon until the age of 50 years, after which there is a steep rise in incidence rates.

Mechanisms responsible for this disease are not known. Among the elderly [2, 3] individuals, recent studies have focused on cell-mediated immunity [4-6].

Results of these studies were variable. Miller [4] found decreased stimulation of lymphocytes in elderly subjects to VZV antigens but not to purified protein derivative or streptokinase-streptodornase. There was also a diminished response to phytohemagglutinin (PHA) and concanavalin A (Con A). Berger et al. [5] reported decreased lymphocyte stimulation to VZV in approximately one-third of the elderly subjects in their study; however, the response to PHA was well preserved. Burke et al. [6] found decreased lymphocyte stimulation to VZV and PHA in individuals past 60 years of age. Skin test (delayed) reactivity to VZV was significantly diminished, but less so than reactivity to PHA.

γ-Interferon (IFN-γ) is a lymphokine thought to be of importance in immunoregulation and resistance to viral infections. Because of the paucity of data on IFN in the elderly, and in order to shed additional light on the mechanisms of VZV reactivation, we sought to determine IFN response in the lymphocytes of elderly subjects to VZV, herpes simplex virus (HSV), cytomegalovirus (CMV), and
two T cell mitogens, PHA and staphylococcal enterotoxin B (SEB).

Materials and Methods

Study subjects. The study population comprised 37 subjects. There were 21 healthy young adults (mean age, 28 ± 1.8 years; median age, 28; range, 27–35) and 16 elderly subjects (mean age, 77 ± 6.6 years; median age, 75.5; range, 68–88). The elderly subjects were in a good state of health, were well nourished, and were not receiving any immunosuppressants. They all resided in a retirement home and were completely self-sufficient. Their complete blood cell counts, liver and renal function tests, and protein and albumin levels were all within normal limits. Thirteen of the 14 young adults had had varicella in childhood. Six of the elderly subjects had had a history of zoster; in all cases, the episode had occurred more than one year before the study.

Antibody determinations. IgG antibody determinations were done by using ELISA kits (Herpelisa®, Varicela,® (Cytomegalia®) from M. A. Bioproducts (Walkersville, Md). Readings were made on a spectrophotometer (MR 580 Microelisa Auto Reader; Dynatech, Santa Monica, Cali) at 405 nm. Results were reported in terms of spectrophotometric units and qualitative interpretations ("positive," "negative") based on linear regression analysis using positive and negative controls, according to the manufacturer’s instructions.

Induction of IFN. Peripheral blood lymphocytes were separated by ficoll-hypaque gradients (Ficoll-Paque®, Pharmacia Fine Chemicals, Piscataway, NJ). Cell viability was determined before each experiment by erythrocin-B dye exclusion test and was uniformly found to be ≥99%. Lymphocytes were washed three times in RPMI 1640 medium and resuspended to a concentration of 5 × 10⁶ cells/ml (final volume) in the same medium, with 15% autologous plasma, in 3-ml vials. (In preliminary experiments, 15% autologous plasma was found to give higher IFN levels than did 30% autologous plasma or fetal bovine serum and human type AB plasma at 15% and 30%). Mitogens and antigen preparations were added in OJ-ml volumes per vial to triplicate cultures. The mitogens consisted of PHA-P (Burroughs Wellcome, Greenville, NC) and SEB (Sigma, St. Louis), both at a final concentration of 1 μg/ml. Viral antigens consisted of CF test reagents obtained from M. A. Bioproducts. The original concentration was ≥10⁴ TCID₅₀/0.2 ml in MRC-5 cells; the concentration by CF antigen was ≥1:8/ml. Viral antigen preparations were inactivated by UV irradiation (with germicidal lamp G8T5; (George W. Gates, Long Island, New York) at a distance of 13 cm for 10 min with constant stirring). No infectivity or cell toxicity was detected after inactivation. Viral antigens were used at final dilutions of 1:100 and 1:1,000.

Mitogen controls consisted of PBS, and viral controls were uninfected tissue culture preparations (M. A. Bioproducts) treated in the same manner as antigen preparations. Mitogen stimulation was carried out for three days and viral stimulation for seven days. Supernates were then harvested for IFN titration and stored at −75 C. The concentrations of mitogens and antigens used and the times of incubation were found in preliminary experiments to give optimal IFN production in young adult control subjects.

IFN titration. IFN levels were determined by 50% yield reduction in an HA assay of encephalomyocarditis virus in human amnion (WISH) cells, as described by Jameson et al. [7]. Antiviral activity was expressed in IU and was standardized by using the National Institutes of Health human α-IFN (HuIFN-α; leukocyte) standard IRP 69/19.

Characterization of IFN. Characterization of IFN was done according to the methods of Preble et al. [8]. IFN standards of HuIFN-α, human β-IFN (HuIFN-β; fibroblast), human IFN-γ (HuIFN-γ; immune) were from the Research Resources Branch, National Institute of Allergy and Infectious Diseases (Bethesda, Md). Specific antisera for neutralization included antibody to HuIFN-γ (Meloy Laboratories, Springfield, Va) and reference antibodies to HuIFN-α and -β (Research Resources Branch). Bovine cells (MDBK [NBL-1]) were obtained from the ATCC (Rockville, Md).

Lability at pH 2 was determined by stepwise addition of 1.0 N HCl until pH 2 was reached (measured by pHydrion®, microfine pH paper; Micro Essential Lab, Brooklyn, NY). The preparation was then incubated at 4 C for 24 hr and neutralized to pH 7.4 by stepwise addition of 1.0 N NaOH. Temperature sensitivity was measured by inactivation at 56 C in a water bath for 60 min.

Statistical analysis. Because of the occurrence of positive skewness in many of the data sets, summary statistics are given in terms of the geometric mean (GM) and the geometric deviation (GD). The GD is obtained by taking the antilog of the SD of log-transformed data. The differences in geometric mean are given in terms of the geometric mean (GM) and the geometric deviation (GD). The GD is obtained by taking the antilog of the SD of log-transformed data. The difference between the young adult and elderly groups was assessed by application of the test to log-transformed data. Comparison of two percentages was performed with a χ² 2 × 2 contingency table. A probability level <.05 indicated statistical significance.

Results

Antibody determinations. More elderly than young adult subjects were seropositive for HSV-1 (81.2% vs. 47.6%; P < .05; table 1). The same was true for CMV (68.7% vs. 33.3%; P < .05). All subjects were seropositive for VZV.

GM levels of antibody to VZV in spectrophotometric units were comparable in the elderly and young adult subjects (.411 vs .382), whereas elderly subjects had signifi-
Table 1. Number of elderly and young adult subjects seropositive to herpesvirus.

<table>
<thead>
<tr>
<th>Subjects (n)</th>
<th>HSV-1</th>
<th>CMV</th>
<th>VZV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Young adult (21)</td>
<td>10 (47.6)</td>
<td>7 (33.3)</td>
<td>20† (100)</td>
</tr>
<tr>
<td>Elderly (16)</td>
<td>13 (81.2)</td>
<td>11 (68.7)</td>
<td>16 (100)</td>
</tr>
<tr>
<td>Young adult vs. elderly</td>
<td>P &lt; .05</td>
<td>P &lt; .05</td>
<td>NS</td>
</tr>
</tbody>
</table>

NOTE. NS = not significant.
* Measured by ELISA.
† Total no. studied was 20.

The data for IFN responses to viral antigens is presented for seropositive subjects only. For HSV, GM titers were statistically significant at both antigen dilutions (260 IU vs. 63 IU, P < .05; 31.8 IU vs. 6.9 IU, P < .01 in young and elderly subjects, respectively). For VZV there was a consistent trend for young adults to have higher IFN titers. This trend reached statistical significance at a 1:1,000 antigen dilution (11.5 vs. 5.8 IU, P < .05). The responses to CMV, however, were not significantly different between the young adult and the elderly groups.

We also sought to identify subjects who were non-responders in herpesvirus-induced IFN-stimulation assays. Twenty young adults had a negative response in six (10.3%) of 58 of such assays; three times as many negative responses were observed in the 16 elderly subjects—14 (29.1%) of 48 (P < .05).

Finally, we characterized the IFNs induced by mitogens and antigens in lymphocytes from selected subjects. IFN induced by PHA and SEB fulfilled all of the characteristics of HuIFN-γ, in that it had a significantly higher titer in human than in bovine cells, was neutralized by antibodies to IFN-γ only, and was pH 2 and heat (56 C for 30 min) sensitive. Virus-induced IFNs appeared to be a mixture of IFN-γ and IFN-α, with IFN-γ characteristics predominating.

Discussion

Herpes zoster is much more common in elderly than in younger individuals, but the reasons for this are poorly understood. Initially it was thought that VZV reactivation occurred when antibody levels declined below a critical point [1]. Several studies (including this one), however,

Table 2. IFN titers in lymphocyte-stimulated supernatants in seropositive elderly and young adult subjects.

<table>
<thead>
<tr>
<th>Mitogen or antigen</th>
<th>Young adult subjects</th>
<th>Elderly subjects</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. tested</td>
<td>GM titer (IU/ml)</td>
<td>GD</td>
</tr>
<tr>
<td>PHA (1 µg/ml)</td>
<td>20</td>
<td>284.3</td>
<td>3.86</td>
</tr>
<tr>
<td>SEB (1 µg/ml)</td>
<td>21</td>
<td>1106.7</td>
<td>4.93</td>
</tr>
<tr>
<td>HSV-1 1:1,000</td>
<td>10</td>
<td>260.0</td>
<td>5.23</td>
</tr>
<tr>
<td>HSV-1 peak response</td>
<td>10</td>
<td>260.0</td>
<td>5.23</td>
</tr>
<tr>
<td>CMV 1:1,000</td>
<td>6</td>
<td>37.4</td>
<td>4.79</td>
</tr>
<tr>
<td>CMV peak response</td>
<td>7</td>
<td>22.2</td>
<td>6.03</td>
</tr>
<tr>
<td>VZV 1:1,000</td>
<td>12</td>
<td>25.6</td>
<td>3.47</td>
</tr>
<tr>
<td>VZV peak response</td>
<td>19</td>
<td>11.5</td>
<td>2.51</td>
</tr>
</tbody>
</table>

NOTE. GM = geometric mean; GD = geometric deviation (see Materials and Methods).
* Young adult vs. elderly subjects.
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have shown levels of antibodies to VZV to be normal in elderly persons [2, 3].

Lymphoproliferative response, as measured by increased uptake of [3H]Tdr into the DNA of lymphocytes stimulated by mitogens or antigens, is considered a good indicator of cell-mediated immunity [9]. Studies using lymphoproliferation have shown that response to mitogens (such as PHA and Con A) in lymphocytes from elderly subjects is diminished [4, 5]. However, it was found that variables such as mitogen concentration [10] or time of incubation may influence the results [11]. Several authors [4-6] have also reported impaired lymphoproliferative response to VZV antigens in elderly as compared with young adult subjects. In the present study we also determined lymphoproliferative responses. In the elderly subjects these responses were uniformly lower for PHA and SEB (P < .05) and for HSV and VZV (not significant) but not for CMV. Because there was no direct correlation between lymphocyte stimulation and IFN levels, these data were not reported.

IFNs are important moieties that can directly suppress viral replication and modulate the immune response [12]. Not much is known about IFN response in the elderly host. We have previously shown diminished serum levels of IFN induced by Newcastle disease virus in old compared with young mice [13]. Miller [4] reported normal IFN levels in response to stimulation of lymphocytes from elderly subjects with VZV antigen. However, only six elderly and six young adult control subjects were studied. Because of the paucity of data on IFN in the elderly host and because of the possible importance of IFNs in the control of herpes zoster [14], we have decided to study the response of elderly subjects' lymphocytes to VZV antigen (as well as to antigens of two other herpesviruses, HSV and CMV).

We have found a significant decrease in the IFN response to VZV and HSV antigens in the elderly subjects. The IFN response to CMV was comparable in seropositive elderly and young adult subjects. The reason for this difference in IFN responses is not readily apparent. This preliminary observation of impaired IFN response (largely γ) to VZV (and HSV) in lymphocytes from elderly subjects may account, in part, for the increased incidence of herpes zoster in elderly persons.

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References


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