Varicella-zoster virus (VZV)-specific cell-mediated immunity (CMI) responses were compared over time following an episode of herpes zoster (HZ) with those of age-, race-, and gender-matched healthy controls (HC) without HZ, using a validated gamma interferon (IFN-γ) enzyme-linked immunospot (ELISPOT) assay. The zoster brief-pain inventory (ZBPI) was used to assess zoster-associated pain. HZ patients (n = 140) had significantly higher IFN-γ ELISPOT responses to VZV antigen than did HC (n = 140). ELISPOT geometric mean count (GMC) responses (with 95% confidence intervals [CI]) for subjects who presented within 72 h were as follows: for HZ patients ≥ 60 years of age, at day 0 the GMC was 110 and at week 2 the GMC was 235; for HZ patients 21 to 59 years of age, at day 0 the GMC was 111 and at week 2 the GMC was 198; for HC ≥ 60 years of age, at day 0 the GMC was 19 and at week 2 the GMC was 18; and for HC 21 to 59 years of age, at day 0 the GMC was 59 and at week 2 the GMC was 56. The mean pain score (95% CI) across age groups at 1 week postrash (n = 106) was 6.0 (5.5, 6.5) and at 2 weeks postrash (n = 119) was 3.5 (2.9, 4.0). The percentage of HZ patients with substantial pain (score ≥ 3) at 6 weeks postrash increased with age from 8% for patients 21 to 49 years of age to 16% for patients 50 to 59 years of age to 22% for patients ≥ 60 years of age. The VZV-specific CMI response was substantially boosted by an episode of HZ, as measured by ELISPOT results. Older adults had lower VZV-specific cellular immunity than younger subjects at baseline, but the boosting effect of HZ was substantial for all age groups. HZ patients experienced considerable zoster-associated acute pain (1 to 2 weeks after rash) pain across age groups, while chronic pain increased with age.

Varicella-zoster virus (VZV) develops a permanent latent association with neurons in spinal and cranial sensory nerve ganglia during primary infection (varicella) (9, 20). Herpes zoster (HZ; shingles), which is the result of reactivation of this latent VZV infection, is characterized by a painful, unilateral, dermatomal, vesicular rash (5, 7, 14). There is a close correlation between the age-related incidence of HZ and the age-related decline in VZV-specific cell-mediated immunity (VZV-CMI) measured by T lymphocyte proliferation assays (using responder cell frequency [RCF] or gamma interferon [IFN-γ] enzyme-linked immunospot [ELISPOT] assays) (10, 21), whereas the level of serum immunoglobulin antibody to VZV remains relatively constant with age (10, 15, 19).

These observations suggest that HZ develops in older individuals because their VZV-CMI falls below some critical threshold that is permissive for clinically apparent VZV reactivation. The pivotal trial with the licensed zoster vaccine was based on this assumed relationship (14). This vaccine, zoster vaccine live (Oka/Merck) (Zostavax; Merck & Co., Inc.), induced VZV-specific antibody and VZV-CMI responses, and the vaccine-induced responses correlated with protection against HZ (10). Zoster vaccine reduced the incidence of HZ and postherpetic neuralgia (PHN) in immunocompetent adults ≥ 60 years of age and diminished the acute and chronic pain associated with HZ (13). Zoster vaccine also prevents HZ in subjects 50 to 59 years of age, and this effect correlates with the vaccine-induced boost in levels of VZV-specific antibody (reference 15 and unpublished data [Merck]).

Understanding the kinetics and age dependence of the VZV-CMI responses to clinically apparent reactivation of VZV (i.e., HZ) and their comparability to vaccine-induced VZV-CMI responses is important for understanding the current value and future use of the zoster vaccine. This probe study determined the kinetics and variability of VZV-specific IFN-γ ELISPOT responses in subjects, in two different age cohorts with acute HZ, and compared them to the VZV-CMI of healthy age-matched subjects without HZ.

MATERIALS AND METHODS

Study population. Healthy subjects ≥ 60 years of age who had resided in the United States for ≥ 30 years and had not had HZ were eligible for the study. Subjects were excluded if they had previously received any VZV-containing vaccine, had been exposed to varicella or HZ within 4 weeks prior to study initiation, were immunosuppressed by illness or medical treatments, had a neoplastic disease, had received corticosteroid treatment within the 4 previous weeks, or had received blood products within 3 months prior to enrollment. The protocol was approved by the Ethical Review Committee of each participating site, and written informed consent was obtained from each subject prior to entry into the study.

Study design. This was a study conducted at 5 sites within the United States between November 2000 and August 2003 to examine the VZV-CMI responses of subjects in the acute or early convalescent phase of HZ. All HZ subjects were clinically diagnosed by the site investigator, and the diagnoses were confirmed by VZV-specific PCR on lesion samples obtained at enrollment (8). The key elements of the diagnosis included a unilateral, dermatomally distributed rash consisting of grouped vesicles...
with signs of inflammation (i.e., erythema) and pain before, during, and/or after the presentation of the rash. If the PCR sample was inadequate, the diagnosis of HZ rested with the site principal investigator. If the PCR sample was confirmed to be negative for VZV, the subject was excluded from all analyses. Enrollment was planned for 50 subjects ≥60 years of age and 30 subjects 21 to 59 years of age with HZ, who were seen by the site staff within 72 h of lesion onset. An additional 50 subjects (25 in each age group) presenting with an HZ rash between 4 and 10 days after lesion onset were enrolled. Each cohort of subjects with HZ was matched with an equal number of healthy control subjects by age, race, and gender. Blood samples were drawn at enrollment (day 0) and at 2 weeks, 6 weeks, and 6 months postenrollment to determine the VZV-specific IFN-γ ELISPOT responses.

Immunogenicity measurements. Blood was analyzed for VZV-CMI responses by IFN-γ ELISPOT assays performed on peripheral blood mononuclear cells (PBMCs) collected and frozen at the study sites. PBMCs were shipped on dry ice to Merck Research Laboratories, West Point, PA, where IFN-γ ELISPOT assays were performed as previously described (11, 17, 18). Spots were enumerated with an ImmunoSpot reader, and data were reported as the net number of VZV-specific IFN-γ spot-forming cells (SFCs) per 10⁶ PBMCs (response to VZV antigen minus response to control antigen). Results of assays for PBMCs processed >24 h after the blood specimen was obtained or with phytohemagglutinin responses of <500 SFCs were not analyzed. The week 0, 3, and 6 samples for a given subject were tested within the same assay run, based upon when samples were received and available for testing. The 6-month samples were tested together when sufficient numbers of 6-month samples had accrued.

Pain surveillance. All HZ subjects were asked to complete the zoster brief-pain inventory (ZBPI) to quantify the intensity and duration of pain at day 0, week 2, week 6, and month 6. The ZBPI is a 9-part questionnaire that assesses the intensity and location of the HZ-associated pain in a subject and measures the effect of pain on their activities of daily living (ADL) (3). The worst pain score responses from the ZBPI were recorded at each visit, and the combined ADL score for an individual was calculated as the average of the scores for the 7 ADL questions on the ZBPI at each visit.

Statistical methods. Geometric mean counts (GMCs) and geometric mean fold rise (GMFR) from day 0 for the VZV-specific IFN-γ ELISPOT assay, and their corresponding 95% confidence intervals (CIs), were summarized for each of the study groups at each time point. Differences in GMCs and GMFRs at day 0 (baseline), week 2, week 6, and month 6 were summarized and compared by disease status (HZ subjects versus controls), by age group (21 to 59 years old versus ≥60 years old), and by stage of HZ outbreak (within 72 h of lesion onset versus days 4 to 10 following lesion onset). The differences in GMCs between HZ subjects and their healthy controls were calculated using a longitudinal regression model that accounted for the correlation among subjects’ ELISPOT measurements at 4 time points.

For the overall comparison of ELISPOT responses between HZ subjects and healthy controls stratified by age categories (21 to 59 years old and ≥60 years old), the study had a power of at least 99% to detect a 2-fold difference, with ~88 evaluable subjects in each group.

Linear-regression analysis was used to explore relationships between pain and ADL at 1 week, 2 weeks, 6 weeks, and 6 months since rash onset. The model used pain as the independent variable and ADL and age as the explanatory variables.

RESULTS

Participant accounting and demographics. Figure 1 indicates that the completion rates were high in all study groups: 87.5% for the 72-h HZ subjects and 97.7% for their healthy controls and 88.5% for the day 4 to 10 HZ subjects and 96.3% for their healthy controls. The lower completion rates in the HZ groups were statistically significant (P value = 0.003), mainly due to the disproportionate loss to follow-up or withdrawal of consent in the HZ groups. Overall, 17 of 140 subjects were enrolled based on the investigator’s clinical diagnosis of HZ due to inconclusive PCR results.

Subjects in the two groups were comparable with respect to baseline characteristics (Table 1). The mean ages at enrollment were 60.3 years for the HZ subjects and 59.1 years for the healthy controls. The overall study population was mostly Caucasian. A higher percentage of females (60%) than males (40%) were enrolled in the cohort seen early after HZ onset. Overall, 93% of all HZ subjects and 94% of all healthy controls had at least one underlying medical condition, and 93% of HZ subjects and 88% of healthy controls were receiving at least one therapy for concomitant diseases at baseline. The most commonly reported medical conditions (frequency > 20% in at least one group) were hypertension (44% of HZ patients and 46% of healthy controls), postmenopause (7% and 26%), and hypercholesterolemia (20% and 7%). Antiviral therapy was used by 83% and 77% of HZ subjects
enrolled within 72 h and 4 to 10 days of HZ onset, respectively. Except for the antiviral therapies, the HZ and control groups were generally balanced with respect to underlying medical conditions and concomitant therapies.

**Immunogenicity.** Table 2 displays the results of GMCs for VZV-specific IFN-γ ELISPOT responses for subjects with acute HZ presenting within 72 h of rash onset and for the age-matched controls. These responses rose significantly in the succeeding 2 weeks after enrollment and remained elevated at 6 weeks after initial post-HZ testing in both age cohorts. The magnitudes of the response in the two age categories of subjects with HZ were similar, whereas the ELISPOT responses were higher in younger than in older healthy controls. To explore the effects of age group and time on ELISPOT responses among the HZ subjects and among the healthy controls, longitudinal models using ELISPOT responses as the response variable and gender, age group, and visit as independent variables were fitted for HZ and healthy controls separately for the time period from day 0 to week 6. Among the HZ subjects, the model showed that the first 2-week interval after HZ onset was a statistically significant factor with respect to GMC ($P_{value}/H110050.0237$). Specifically, week 2 GMC was statistically higher than day 0 GMC ($P_{value}/H110050.0108$), but the difference in GMCs between week 2 and week 6 was not significant ($P_{value}/H110050.0554$). Furthermore, this longitudinal analysis showed that age group was not a statistically significant factor for GMC among HZ subjects ($P_{value}/H110050.9586$), supporting the observation repre-

### TABLE 1 Demographics

| Parameter       | Value at indicated presentation time | 4 to 10 days after onset |
|-----------------|--------------------------------------|-------------------------|
|                 | Within 72 h of onset                  | Controls (N = 86b)      |                           |
|                 | HZ subjects (N = 88)                  | Controls (N = 86b)      |                           |
|                 | HZ subjects (N = 52)                  | Controls (N = 54c)      |                           |
| Gender          |                                      |                         |                           |
| Male            | 30                                    | 31                      | 24                       |
|                | 34.1                                  | 36.0                    | 46.2                     |
| Female          | 58                                    | 55                      | 28                       |
|                | 65.9                                  | 64.0                    | 53.8                     |
| Age (yr)        |                                      |                         |                           |
| 21 to 59        | 31                                    | 29                      | 28                       |
| Mean            | 35.2                                  | 33.7                    | 53.8                     |
| SD              | 11                                    | 12                      | 11                       |
| Range           | 23 to 59                              | 23 to 59                | 22 to 59                 |
| Mean            | 74                                    | 74                      | 76                       |
| SD              | 8                                     | 6                       | 6                        |
| Range           | 60 to 95                              | 60 to 96                | 65 to 87                 |
| Race            |                                      |                         |                           |
| White           | 66                                    | 69                      | 40                       |
|                | 75.0                                  | 80.2                    | 76.9                     |
| Hispanic        | 8                                     | 6                       | 8                        |
|                | 9.1                                   | 7.0                     | 15.4                     |
| Black           | 10                                    | 7                       | 3                        |
|                | 11.4                                  | 8.1                     | 5.8                      |
| Other           | 4                                     | 4                       | 1                        |
|                | 4.5                                   | 4.7                     | 1.9                      |
|                | n                                     | n                       | n                        |
| Age group (yr)  |                                      |                         |                           |
| 21 to 59        |                                      | 110.5                   | 58.2                     |
| Day 0a          | 23                                   | 197.1                   | 56.1                     |
| Wk 2a           | 21                                   | 161.3                   | 42.2                     |
| Wk 6a           | 20                                   | 91.7                    | 96.8                     |
| Mo 6            | 19                                   | 139.8                   | 22.0                     |
|                | 45                                   | 81.3                    | 39.1                     |
| ≥60             |                                      | 113.6                   | 50                       |
| Day 0b          | 49                                   | 242.9                   | 45                       |
| Wk 2b           | 48                                   | 242.9                   | 45                       |
| Wk 6b           | 44                                   | 139.8                   | 47                       |
| Mo 6            | 45                                   | 81.3                    | 49                       |

**a** N, number of all clinically diagnosed HZ subjects or controls; n, number of subjects contributing to each category; other, Asian, Native American, and multiracial.

**b** The control group included one subject 20 years of age.

**c** The control group included one subject 19 years of age.

### TABLE 2 VZV-specific IFN-γ ELISPOT responses in HZ subjects who presented within 72 h of rash onset

| Age group (yr) | Endpoint | Value for indicated group | Estimated HZ/control GMC ratio (95% CI) |
|----------------|----------|----------------------------|----------------------------------------|
| 21 to 59       | Day 0a   | n GMC                      | 1.9 (0.77, 4.68)                        |
|                | Wk 2a    | 21 197.1                   | 3.5 (1.49, 8.26)                       |
|                | Wk 6a    | 19 161.3                   | 3.8 (1.81, 8.10)                       |
|                | Mo 6     | 20 91.7                    | 0.9 (0.53, 1.69)                       |
| ≥60            | Day 0b   | 49 113.6                   | 5.7 (2.75, 11.91)                      |
|                | Wk 2b    | 48 242.9                   | 13.2 (6.24, 27.89)                     |
|                | Wk 6b    | 44 139.8                   | 6.4 (3.29, 12.35)                      |
|                | Mo 6     | 45 81.3                    | 2.1 (1.18, 3.66)                       |

**a** Confirmed PCR VZV-negative HZ subjects were excluded from the analysis. Estimated GMC responses, GMC ratios, and 95% CIs were determined on the basis of the use of a longitudinal regression model. N, number of subjects enrolled; n, number of subjects contributing to the analysis at the respective time point in each group.

**b** Samples from a given subject were tested within the same assay run.
sented in Table 2 that HZ elevated ELISPOT responses to similar levels in the two age groups. Among the healthy control subjects, the model showed that the younger age group had a statistically significantly higher GMC (P value < 0.001).

Table 2 shows that the GMC responses in the control group were stable from day 0 to week 6 but were higher at month 6. The assays of blood samples from day 0, week 2, and week 6 were conducted within the same assay run, while those from month 6 were assayed in a separate run. Table 2 also shows the GMC responses in the HZ subjects relative to their corresponding healthy controls. The GMC ratios (HZ/controls), which were estimated from longitudinal models for each time point, support the finding that the ELISPOT responses in both the young and older subjects were elevated by HZ compared with the responses of their corresponding healthy uninfected controls and that this elevation remained until the week 6 time point. The cell-mediated immune response as measured by the ELISPOT assay appears to have declined 6 months after rash onset for all age groups.

Table 3 displays similar data from HZ subjects enrolled 4 to 10 days after HZ rash onset along with data from healthy controls. The data indicate that the peak responses had occurred in both age groups during the longer interval between rash and enrollment. An HZ-related boost persisted at least 6 weeks in both age groups with HZ. Data for healthy controls for each age group were similar to those presented in Table 2.

Pain and activities of daily living. Summaries of the worst pain score and ADL by time since HZ onset are provided in Table 4 and Table 5, respectively. Noticeable interference with ADL was observed for 2 weeks after the onset of HZ rash, which had mostly disappeared by 6 weeks after HZ. Linear-regression models

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**TABLE 3 VZV-γ IFN-γ ELISPOT assay responses among HZ subjects who presented 4 to 10 days after rash onset**

| Endpoint and assay | Time point postenrollment | Value for indicated group |
|--------------------|---------------------------|---------------------------|
|                    | HZ subjects (N = 52) | Controls (N = 54) |
|                    | n | Observed response (95% CI) | n | Observed response (95% CI) |
| 21 to 59 yr GMC    | Day 0 | 21 | 223.3 (119.6, 417.1) | 25 | 63.4 (33.2, 121.2) |
|                    | Wk 2 | 24 | 111.2 (55.3, 223.2) | 19 | 66.8 (29.2, 152.9) |
|                    | Wk 6 | 23 | 89.5 (58.3, 137.2) | 20 | 26.3 (11.0, 62.8) |
|                    | Mo 6 | 21 | 51.1 (23.3, 112.3) | 26 | 52.3 (20.9, 131.0) |
| ≥60 yr GMC         | Day 0 | 16 | 247.9 (119.4, 515.0) | 20 | 10.5 (4.0, 27.9) |
|                    | Wk 2 | 15 | 134.3 (61.8, 291.8) | 18 | 15.9 (5.1, 49.5) |
|                    | Wk 6 | 16 | 79.0 (42.1, 148.2) | 16 | 12.8 (5.9, 27.7) |
|                    | Mo 6 | 18 | 46.2 (24.5, 87.1) | 21 | 19.1 (7.6, 48.0) |
| ≥60 yr GMFR        | Wk 2 | 20 | 0.7 (0.3, 1.5) | 18 | 1.2 (0.8, 1.7) |
|                    | Wk 6 | 19 | 0.6 (0.3, 1.0) | 16 | 0.5 (0.2, 1.2) |
|                    | Mo 6 | 17 | 0.3 (0.1, 0.9) | 22 | 1.1 (0.6, 1.9) |

**TABLE 4 Summary of worst pain by time since rash onset**

| Age group and time point since rash onset | N | Mean worst pain score (95% CI) | No. of subjects with worst pain score ≥3 (%) |
|------------------------------------------|---|--------------------------------|-------------------------------------------|
| 21 to 59 yr Wk 1                        | 39 | 5.8 (5.0, 6.6) | 35 (89.7) |
| 21 to 59 yr Wk 2                        | 47 | 2.7 (1.8, 3.5) | 22 (46.8) |
| 21 to 59 yr Wk 6                        | 44 | 0.9 (0.3, 1.6) | 5 (11.4) |
| 21 to 59 yr Mo 6                        | 42 | 0.4 (−0.0, 0.9) | 2 (4.8) |
| ≥60 yr Wk 1                             | 67 | 6.1 (5.4, 6.8) | 57 (85.1) |
| ≥60 yr Wk 2                             | 72 | 4.0 (3.3, 4.7) | 48 (66.7) |
| ≥60 yr Wk 6                             | 64 | 1.9 (1.3, 2.5) | 19 (29.7) |
| ≥60 yr Mo 6                             | 69 | 0.3 (0.0, 0.6) | 4 (5.8) |

**TABLE 5 Summary of combined ADL ZBPI scores by time since rash onset**

| Age group (yr) | Time point since rash onset | n | Mean combined ADL score (95% CI) |
|----------------|-----------------------------|---|--------------------------------|
| 21 to 59        | Wk 1                        | 37 | 3.5 (2.6, 4.5) |
|                 | Wk 2                        | 47 | 1.8 (1.0, 2.6) |
|                 | Wk 6                        | 44 | 0.7 (0.1, 1.5) |
|                 | Mo 6                        | 41 | 0.2 (0.1, 0.4) |
| ≥60             | Wk 1                        | 67 | 3.6 (3.0, 4.3) |
|                 | Wk 2                        | 72 | 2.4 (1.8, 3.0) |
|                 | Wk 6                        | 64 | 1.3 (0.8, 1.7) |
|                 | Mo 6                        | 68 | 0.1 (0.0, 0.3) |

**TABLE 5 Summary of combined ADL ZBPI scores by time since rash onset**

| Age group (yr) | Time point since rash onset | n | Mean combined ADL score (95% CI) |
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| 21 to 59        | Wk 1                        | 37 | 3.5 (2.6, 4.5) |
|                 | Wk 2                        | 47 | 1.8 (1.0, 2.6) |
|                 | Wk 6                        | 44 | 0.7 (0.1, 1.5) |
|                 | Mo 6                        | 41 | 0.2 (0.1, 0.4) |
| ≥60             | Wk 1                        | 67 | 3.6 (3.0, 4.3) |
|                 | Wk 2                        | 72 | 2.4 (1.8, 3.0) |
|                 | Wk 6                        | 64 | 1.3 (0.8, 1.7) |
|                 | Mo 6                        | 68 | 0.1 (0.0, 0.3) |

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* N, number of subjects (either investigator-diagnosed HZ subjects or healthy controls) enrolled; n, number of subjects contributing to each category; GMC, geometric mean count; GMFR, geometric mean fold rise (from day 0).
showed that since rash onset was a significant determinant of worst pain or ADL interference (P value < 0.001). In the first week after rash onset, scores were comparable. Higher pain scores in the group of subjects ≥60 years of age by week 2 corresponded to the increased impact on ADL. Higher pain was associated with higher ADL interference, and this relationship was supported by linear-regression analysis of the relationship between worst pain score and ADL interference (P value < 0.001 at each time point since rash onset).

**DISCUSSION**

The VZV-CMI measurements from the controls for the two age cohorts (21 to 59 years versus ≥60 years) indicate that younger individuals had significantly higher responses, as measured by IFN-γ ELISPOT assay, than the older individuals. This observation supports an important aspect of immune senescence which has been demonstrated in other cross-sectional studies performed using other measures of VZV-CMI (1, 2, 12, 21). The IFN-γ ELISPOT responses were substantially boosted by an episode of HZ. Table 2 indicates that the magnitudes of the responses of the two age cohorts were comparable at each time point, indicating that older individuals, in spite of depressed numbers of circulating effector cells in the base state, can develop a strong VZV-CMI response by the time skin lesions appear. The boosting effect of HZ was maximal prior to 6 weeks after rash onset. This finding is consistent with another study that described a peak ELISPOT response between 1 and 3 weeks after the onset of HZ rash (22). The HZ-induced boost in that study and in the current study was declining by 6 weeks after HZ onset and approximated levels observed in uninfected age-matched controls by 6 months after HZ onset. The kinetics of the ELISPOT response after HZ correlates with cessation of new lesion formation and the pattern of healing observed with typical HZ.

Based on comparison with controls, VZV-CMI responses in subjects with HZ were evident at the time of presentation—as soon as within 4 days of rash onset. Additionally, levels of pain in the acute phase were similar in younger and older patients. These observations are consistent with clinical evidence that VZV-induced neuronal damage occurs early in HZ, as reflected in prodromal signs and symptoms (5) and significant ganglionitis and VZV antigen found in the sensory ganglion very early after HZ onset (4, 6, 16). These findings suggest that the zoster vaccine protects older vaccinees shortly after administration, which is consistent with observations that a protective effect was noted within the 30-day postvaccination period during the definitive trial of the current vaccine (13).

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S. K. Tying and M. J. Levin were responsible for subject enrollment, data collection, data interpretation, and manuscript preparation. J. G. Smith, J. Xu, I. S. F. Chan, and J. L. Silber were responsible for study concept/design, data analysis/interpretation, and manuscript preparation. J. E. Stek, M. Pagnoni, and J. Parrino were responsible for data analysis/interpretation and manuscript preparation.

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