Herpes zoster (HZ), or shingles, is caused by reactivation of an endogenous varicella-zoster virus (VZV) infection that has persisted in latent form within sensory ganglia following an earlier attack of varicella [1−2]. Both the incidence and severity of HZ increase markedly with increasing age, and the majority of episodes of HZ occur in persons >60 years of age [1−4]. The factors associated with the increased risk of HZ in older adults and the mechanisms responsible for maintaining VZV in the latent state are not known. Nevertheless, there is much evidence that cell-mediated immunity plays a central role in limiting the occurrence of HZ and its complications [1, 2, 5]. The increase in the incidence and severity of HZ in elderly persons is correlated with an age-related decline in cell-mediated immunity to VZV [5−10], and conditions that compromise cellular immunity are associated with a marked increase in the age-specific incidence of HZ and its complications [2, 5, 10]. In contrast, serum antibody levels are maintained in older adults, and deficits in humoral immunity are not associated with an increase in the incidence or severity of HZ [2].

In view of recent evidence that the immune system is integrated with other homeostatic mechanisms that are ultimately regulated by the brain [11, 12], the influence of biobehavioral factors on the incidence of HZ and on VZV-specific immune responses deserves attention. While there are some data linking major depression and recurrences of herpes simplex [13], similar observations related to HZ are limited. Schmader et al. [14] have shown that self-reported psychologic stress correlates with the occurrence of HZ [14], but we are not aware of studies of the impact of major depression or psychologic stress on VZV-specific cellular immunity.

The immunologic consequences of major depression and their possible clinical relevance to infectious diseases have not been delineated [15, 16]. Major depression has been associated with alterations in the distribution of T cell subsets and with declines in nonspecific measures of immune function, such as NK cell activity and mitogen-induced lymphocyte proliferation [15−17], but depression has also been associated with immune activation [18]. The clinical significance of these immunologic findings is uncertain because the in vitro assays used were nonspecific and thus not directly relevant to specific disease end points [15].

To address these issues, we examined the effect of major depression on VZV-specific cellular immunity. The frequency of cells in the peripheral blood capable of proliferating in response to VZV antigens (VZV-specific responder cell frequency [RCF]) was determined in patients with major depression and in age- and sex-matched normal controls. In addition, we evaluated the VZV-specific RCF in a group of older adults to determine whether the decline in RCF observed in major depression was comparable in magnitude to that typically found in older persons who are known to be at increased risk of developing HZ.

Methods

Subjects. A total of 53 subjects were recruited for this study. The depressed patient group (n = 11) was selected on the basis...
of DSM-IV criteria for major depressive disorder [19], and they were studied though the University of California, San Diego, Mental Health Clinical Research Center (MHCRC). To include a broad range of depressed subjects, we studied patients hospitalized in the MHCRC unit of the San Diego VA Medical Center and outpatients entering research treatment protocols at the MHCRC.

Controls (n = 42) were recruited from the local San Diego community by means of flyers and advertisements in local newspapers and university newsletters. Many of the older subjects included in this study were individuals who had agreed to participate in a trial of live attenuated OKA/Merck VZV vaccine and who were evaluated prior to vaccination. For comparison with the depressed subjects, a subset of age- and sex-matched controls (n = 11) was tested within 14 days of its depressed counterparts.

All subjects underwent a psychiatric diagnostic interview, the Structured Clinical Interview for DSM-IV [20]. For the depressed subjects and normal controls, diagnoses were assigned in a consensus meeting of a psychiatrist (M.L.) and research fellow (K.H.A.). All depressed subjects fulfilled DSM-IV criteria for current major depressive disorder [19]. All controls fulfilled criteria for being classified as never mentally ill [19]. Depressed subjects and controls were medically stable and had no history of chronic obstructive lung disease, diabetes mellitus, or malignancy. All subjects were seropositive for VZV and had a history negative for HZ.

None had a history of recent (<2 weeks) viral infection.

Consistent with the procedures of the MHCRC, only depressed subjects who were free of psychotropic medications or who were able to discontinue psychotropic medication use for ≥2 weeks without risk of becoming suicidal were eligible for entry into ongoing research protocols. Consequently, none of the depressed subjects had received psychotropic medications (antidepressants or anxiolytics) within 2 weeks of blood testing, and none of the subjects were using medications, such as corticosteroids, that are known to alter immune responses. Control subjects were also free of psychotropic and immunosuppressive medications. The depressed subjects and the subset of age- and sex-matched controls also underwent a physical examination and a laboratory screening examination (i.e., complete blood cell count and chemistry panel) and were found to be in good physical health and to have laboratory results within normal limits.

Procedures. On the morning that blood samples were obtained, an intravenous heparinized catheter was placed into a fore- arm vein. Changes in activity can result in the release of catecholamines, redistribution of T cell subsets, and altered in vitro immune responses [21]; therefore, after catheter placement, subjects remained supine for 20 min in a quiet room before 40-mL samples of peripheral blood were obtained. We have found that this procedure results in stable, resting baseline levels of plasma catecholamines [22]. All blood samples were obtained between 8 and 10 A.M. to control for circadian variation in the number of circulating T lymphocytes. Assays of VZV-specific RCF were initiated within 2 h of sample collection.

Of the 40 mL of blood from each subject, 10 mL was allowed to clot to yield autologous serum; the remaining 30 mL was heparinized. The heparinized samples were diluted with an equal volume of cold Hanks’ balanced salt solution (HBSS) and centrifuged on HistoPaque (Sigma, St. Louis) gradients for 25 min at 750 g. Cells banding at the interface (peripheral blood mononuclear cells [PBMC]) were aspirated, washed twice in HBSS, and adjusted to a cell concentration of 10⁶ cells/mL in RPMI 1640 supplemented with 10% autologous serum.

Details of the limiting dilution assay used to determine the RCF have been published [9, 23]. In brief, 96-well, round-bottom tissue culture plates (catalog no. 25850; Corning, Corning, NY) were prepared in advance by placing 20 μL of a 1:200 dilution of VZV or control antigen (prepared by glycine buffer, pH 9.0 extraction of VZV-infected or uninfected [control] human embryonic lung fibroblasts) in each well. Plates were stored at −20°C.

Replicate wells containing VZV antigen or control antigen (24 replicate wells of each per dilution) were inoculated with serial 2-fold dilutions of PBMC in 100 μL of RPMI 1640 medium supplemented with antibiotics and 10% autologous serum. This dilution process yielded VZV antigen and control plates with PBMC concentrations of 100,000, 50,000, 25,000, and 12,500 PBMC/well. For subjects who were <60 years old, the dilution range was extended down to 3125 PBMC/well in order to quantify high RCF levels without extrapolation. After 10 days of culture at 37°C, each well was radiolabeled with 0.25 mCi methyl [3H]thymidine (6.7 Ci/μmol, catalog no. 2406605; ICN, Costa Mesa, CA) for 6 h. Plates were then frozen at −20°C and subsequently thawed and harvested on a 96-well harvester (Skatron Instruments, Sterling, VA), and the counts per minute (cpm) were determined with a beta plate reader (Packard, Downers Grove, IL).

The data file was imported directly into a spreadsheet to calculate the mean cpm (±SD) incorporated for each set of 24 replicate wells stimulated with control antigen. Wells stimulated with VZV antigen with incorporated cpm greater than the mean ± 3 SD of cpm incorporated into wells containing the same number of PBMC stimulated with control antigen were designated “responder wells” and assumed to contain ≥1 VZV-specific responder cell [23]. The percentage of nonresponder wells was determined for each set of 24 replicates, and the log of the percentage of nonresponder wells was plotted against the number of PBMC per well. The RCF, defined as the reciprocal of the number of PBMC required to yield 1 VZV-specific responder cell, was determined by interpolating the number of PBMC per well at which 37% of VZV antigen–stimulated wells were nonresponders [23]. For subjects <60 years old, RCF values were within the dilution range, and high RCF levels identified in some of these subjects were quantified without extrapolation. However, for 8 of the 39 subjects who were ≥60 years old, RCF values were outside the dilution range. Similar overall results were obtained when these high RCF values were set at the limit of the dilution range and when they were extrapolated to values beyond the dilution range. Therefore, in the analyses reported here, these RCF values were quantified at the limit of the dilution range.

To determine whether depression was associated with an alteration in VZV-specific RCF, we compared the depressed subjects with a subset of controls who were matched on the basis of age, sex, and date of assay. Independent two-tailed t-tests were used to evaluate differences in RCF between the 2 groups. To determine whether the reduction in RCF in depressed subjects was comparable in magnitude to that found in older adults, RCF results from the entire study sample (n = 53) were analyzed. The controls were stratified on the basis of age (<60 and ≥60 years old), and analysis of variance was used to assess differences in RCF values between the groups.
Results

The mean age of the depressed subjects was 51.3 ± 15.7 years (range, 32–77) and that of the matched controls was 51.4 ± 17.2 years (range, 28–79) (t = 0.01, P = .99). There were 7 men and 4 women in each group. As shown in figure 1, VZV-specific RCF was significantly lower in the subjects with major depression (mean RCF = 3.3 ± 2.9 responder cells/10⁵ PBMC) than in matched controls (mean RCF = 10.2 ± 8.1 responder cells/10⁵ PBMC; t = 2.7, P < .02).

To assess the possible clinical significance of the reduced RCF in the depressed subjects, we compared their RCF values with those in the normal older subjects. The results are shown in figure 1. As reported previously, VZV-specific RCF values decline with increasing age [9]. Seven nondepressed subjects who were <60 years old (mean, 40.1 ± 9.3; range, 28–57) had a mean RCF of 12.7 ± 9.4 responder cells/10⁵ PBMC, whereas 35 other normal subjects who were ≥60 years old (mean, 71.2 ± 6.7; range, 60–80) had a mean RCF of 4.1 ± 2.8 responder cells/10⁵ PBMC (F = 13.5, df = 2.52; P < .001). Eleven depressed subjects with a mean age of 51.4 ± 8.2 years (range, 28–79) had a mean RCF of 3.3 ± 2.9 responder cells/10⁵ PBMC, a level comparable to that of normal subjects >20 years their senior.

Discussion

These findings suggest that major depression is associated with a marked decline in VZV-specific cellular immunity, as measured by the frequency of PBMC capable of proliferating in response to VZV antigens (RCF). Furthermore, consistent with prior observations [6–10], the present study documents an age-related decline in VZV-specific cellular immunity in nondepressed adults. While the results reported here do not directly link depression with an increase in VZV reactivation and in the incidence of HZ, comparable declines in VZV-specific cellular immunity observed in older adults have been correlated with a significant increase in the incidence of HZ and its complications [5–10]. The levels of VZV-specific RCF observed in our depressed subjects are similar to those observed in normal adults >60 years of age, in whom the incidence of HZ is more than double that in younger nondepressed adults.

Animal models have yielded compelling evidence that behavioral stressors may impact viral diseases, such as herpes simplex, influenza, and coxsackievirus infections, via alterations in immune function [24]. In contrast, there is a paucity of clinical data addressing the confluence of behavioral, immunologic, and outcome variables in the same individual at the same time [25]. Psychosocial stress appears to be associated with reduced immunologic control of latent herpesviruses (Epstein-Barr virus, herpes simplex virus, and cytomegalovirus), as evidenced by elevated antibody titers [25]. Cohen and colleagues [26, 27] have found psychological stress to be associated with increased rates of respiratory infection after experimental inoculation of common cold viruses, and Glaser et al. [28] and Kiecolt-Glaser et al. [29] have reported that psychological stress is associated with decreased immune responses to hepatitis B and influenza vaccination. However, the association between psychosocial factors, immunity, and clinical outcomes is relatively unexplored in older adults, in whom age-related decrements in immune function might be expected to have significant clinical consequences. Given the prevalence of de-

Figure 1. VZV responder cell frequency (mean ± SE) in subjects with major depression (n = 11) and in age- and sex-matched normal controls (n = 11). Mean age was 51.3 ± 15.7 years (range, 32–77) for depressed subjects and 51.4 ± 17.2 years (range, 28–79) for controls. PBMC = peripheral blood mononuclear cells.
pression in older adults, which may be as high as 5% in women and 2% in men [30], the chronic relapsing and remitting course of depressive illness in the elderly, and the well-recognized impact of depression on functional status and on the outcome of numerous medical conditions, it is important to confirm and extend the present observations.

The number of subjects that we studied who had major depression was small; thus, conclusions from these data require cautious interpretation. The mechanism responsible for the decline in cellular immunity to VZV in depressed patients has not been determined, and it is not clear that the mechanism is the same as that responsible for the observed decline in VZV-specific RCF in normal elderly adults. Further investigation will be required to address these issues and to determine whether the results reported here can be generalized to other populations, such as depressed elderly adults. Finally, assessing the impact of the severity of depression symptoms and of symptomatic treatment of depression on VZV-specific cellular immunity could have therapeutic implications. For example, severity of depressive symptoms, especially insomnia and EEG sleep disturbance, is correlated with the magnitude of reductions in NK cell activity and lymphocyte proliferation observed in depressed subjects, and effective psychopharmacologic treatment is associated with normalization of these in vitro measures of immune function [16].

Despite the limitations of the present study, our finding that major depression is associated with a marked reduction in VZV-specific cellular immunity suggests that depression may be a significant risk factor for HZ that is independent of age. However, none of the depressed subjects developed HZ during the course of the study. This is not unexpected in view of the low annual incidence of HZ (<0.5 cases/1000 persons per year in persons 55 years of age [1, 3, 4]), the small number of depressed subjects studied, the cross-sectional study design, the criteria used for the diagnosis of major depression (i.e., the presence of depressive symptoms for 1 month prior to immunologic evaluation), and the initiation of antidepressant medication after immunologic evaluation. A prospective longitudinal study will be required to determine whether this alteration in VZV-specific cellular immunity that we observed leads to an increase in the incidence of HZ in persons with major depression.

References