STRESS MEDICINE, VOL. 13: 23–29 (1997)

CHANGES IN CIRCULATING LYMPHOCYTE NUMBERS FOLLOWING EMOTIONAL DISCLOSURE: EVIDENCE OF BUFFERING?

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SUMMARY

In order to assess whether disclosure of emotions through writing about upsetting or traumatic events resulted in changes in blood-associated immune variables over time, healthy volunteers were randomly assigned to write about either emotional issues or trivial topics for 4 consecutive days. Circulating lymphocytes and T lymphocyte subsets (CD4 (helper), CD8 (cytotoxic/suppressor)) as well as a variety of standard hematological markers were measured following the writing intervention and compared with baseline values. In two separate studies ($N = 40$ and $N = 38$), there were reproducible significant differences between the emotional disclosure and control writing groups immediately following the intervention in CD4, CD8 and total circulating lymphocyte numbers but not in CD4/CD8 ratios or any other hematological variables. Circulating lymphocyte numbers in the emotional writing group stayed relatively constant over the time-course of the studies, suggesting that the difference between the groups was due to a transient elevation in postwriting blood lymphocyte numbers in the control group. Self-evaluations immediately before and after each writing session confirmed that the intervention was stressful for subjects in the emotional disclosure group but the effects on circulating lymphocytes were not attributable purely to anxiety-related factors. The results extend our previous observation of changes in immune reactivity following a writing intervention and indicate that the group differences are the result of fluctuations over time in the control group but relative stability in the emotional disclosure group. It is conceivable that such 'buffering' of temporal immune variation might be influential in the health-promoting effects of emotional disclosure.

KEY WORDS — psychoneuroimmunology; emotion; circulating lymphocytes; disclosure; immunology; T cells

A considerable body of literature in psychoneuroimmunology has explored the relationship between immune function and perceived stress as a result of life events,[1–3] but to date there has been relatively little work focusing on how such effects might be modulated by deliberate intervention. In a previous study, we assessed whether emotional expression of traumatic experiences influenced the immune response to hepatitis B vaccination.[4] We found that subjects assigned to write about personal traumatic events over 4 consecutive days developed significantly higher antibody levels against hepatitis B vaccine over the subsequent 6 months than did subjects assigned to write about trivial topics. This finding suggests that, as well as having general health benefits,[4–8] emotional disclosure may influence immune responsiveness. In support of this, significant differences have been observed in the mitogen-induced, blastogenic responses of T lymphocytes between emotional expression and control writing groups 6 weeks after the intervention.[5]

Other research had found that expression of emotions (particularly negative emotions) in an experimental context has been associated with transient changes in proliferative responses (mitogenesis) of blood T lymphocytes[9,10] and with small elevations in natural killer cell activity.[11]

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CCC 0748–8386/97/010023–07

Repression of negative affect, as measured by a laboratory task involving disclosure of emotional material, correlated with higher serum antibody titers in subjects with latent Epstein-Barr virus (EBV) infection[12] indicative of poorer cell-mediated control of the virus and consequent reactivation. Moreover, subjects assigned to write or talk about stressful events displayed significantly decreased EBV antibody titers over a 4-week period when compared with subjects assigned to the trivial disclosure control group,[7] and ability to become absorbed in the disclosure process was predictive of antibody decrements.[13]

Our previous study[4] also indicated that among the immune changes were effects on helper T lymphocyte numbers. There was a significant difference in the numbers of circulating T helper (CD4) lymphocytes between the traumatic disclosure and trivial disclosure groups the day after the 4 days of writing but this difference was not evident 1 month later. In view of the central role played by T helper lymphocytes in the induction of immune responses we have investigated this effect further. On theoretical grounds,[14,15] it might be expected that integrating or making sense of emotional experiences by constructing a more coherent framework through the disclosure process would have an effect of buffering or stabilizing fluctuations in immune variables over time. Here we compare the results of two separate emotional disclosure studies in which reproducible differences were observed in circulating lymphocyte numbers between traumatic disclosure and control groups following the intervention consistent with this expectation.

METHODS

Subjects

Two studies were conducted a year apart during the winter months of May–August at a time of the year when there were no major university examinations. The first study (aspects of which were reported previously[4]) involved 40 medical student volunteers comprising 19 women and 21 men with an average age of 21.5 years (SD = 2.4). One subject was an extreme outlier in virtually all blood variables and data from this subject have been excluded from the analysis. Thirty-eight medical students (22 women and 16 men with a mean age of 20.1 years, SD = 1.3) volunteered for the second study. All subjects completed the study and received NZ$50 at the time.

Experimental design

The studies were conducted with informed consent and ethical committee approval. On entry to the studies, subjects completed a questionnaire which, as well as demographic data, included the following scales: Life Orientation Test (which measures dispositional optimism),[16] Sense of Coherence Scale (which addresses the meaningfulness, comprehensibility and manageability of a person's life),[17] Positive and Negative Affect Scale (trait measures)[18] and a physical symptom checklist.[19] Subjects were randomly assigned to either an emotional writing or a control writing group and participated in the writing intervention on each of 4 consecutive days immediately preceding the day of the third blood sample. All subjects wrote for 20 minutes each day in a small, darkened basement room using a personal computer following a procedure outlined previously.[4] Instructions to subjects in the emotional writing group included:

> During each of the 4 writing days I want you to write about the most traumatic and upsetting experiences of your whole life. You can write on different topics each day or on the same topic for all 4 days. The important thing is that you write about your deepest thoughts and feelings. Ideally, whatever you write about should deal with an event or experience that you have not talked with others about in detail.

Subjects in the control group were instructed to write on different aspects of their use of time on each of the 4 days. They were asked to write about the following topics on successive days: what they had done in the previous 24 hours, what their plans were for the next 24 hours, the next week and the following 12 months. Each day in their writing, subjects in the control group were asked to write in a purely descriptive and objective way with minimal use of emotions. The emotional writing group comprised nine males and 11 females (study 1) and 10 males and nine females (study 2), while the control group had 12 males and eight females (study 1) and six males and 13 females (study 2), which was not significantly different from the expected distribution ($\chi^2 = 0.85$, NS).

Before and after writing assessments

Brief questionnaires, scored on unipolar seven-point scales, were administered immediately before and after each writing session to assess negative

mood factors (nervous, sad, guilty, fatigued, constrained and anxious), positive mood factors (happy, contented) and physical symptoms (racing heart, upset stomach, headache, dizziness, shortness of breath, cold hands, sweaty hands, pounding heart). After each session subjects also completed a writing evaluation measure which asked them to report the extent to which their writing was personal, meaningful and revealing of their emotions and how much they had held back from previously discussing this material with others.

Blood samples, hematological and lymphocyte surface markers

In study 1, blood samples were taken on the day after the 4 days of writing and then 1, 4 and 6 months later. In study 2, blood samples were taken once a week for 5 consecutive weeks such that two samples were taken before the writing and the third sample corresponded to the day after the 4 days of writing (ie at the same time as the first blood sample in study 1). All blood was drawn into EDTA (anticoagulant) tubes on a Friday between 8.00 am and 11.00 am. Standard hematological markers relating to white blood cells, red blood cells and platelets, together with white blood cell differential counts, were determined using a Bayer Technicon H1 Hematology Analyzer. Proportions of mononuclear cells in the blood bearing the markers CD4 (T helper lymphocytes) and CD8 (T cytotoxic/suppressor lymphocytes) were determined using flow cytometry in a Coulter EPICS Profile II Analyzer with fluorescein-antiCD4 and rhodamine-antiCD8 monoclonal antibodies (Coulter). Absolute numbers of CD 4 and CD8 cells were calculated using these proportions and lymphocyte concentrations from the hematological screen.

RESULTS

Subject ratings

Subject evaluations of their writing each day revealed close similarities between the two studies. Subjects in the emotional disclosure groups rated their writing to be more personal ($F(1,76) = 105.6$), more meaningful ($F(1,76) = 82.1$), to reveal more emotions ($F(1,76) = 250.7$), to cover topics they had held back from discussing ($F(1,76) = 57.4$) but that they wanted to tell others about ($F(1,76) = 32.2$) (all $ps < 0.01$). Further, subjective experience of negative and positive mood and physical symptoms before and after writing also showed significant differences between the two writing conditions and strong similarities between the two studies (Fig. 1), with subjects in the emotional writing group experiencing significantly more negative mood ($F(1,76) = 29.6$) and physical symptoms ($F(1,76) = 21.5$) and less positive mood ($F(1,76) = 18.7$) after writing (ps all < 0.01).

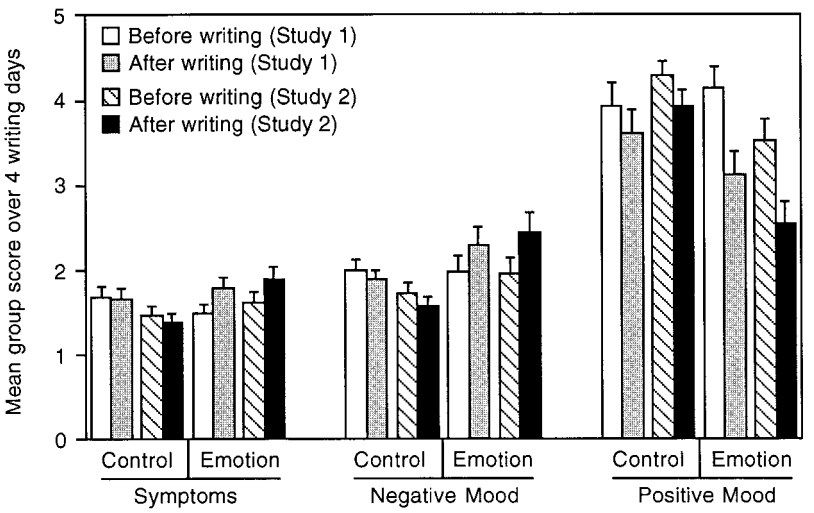

Fig. 1 — Negative and positive mood and physical symptoms ratings of subjects in control and emotional writing groups (group mean + SE) immediately before and after each of the four writing sessions. Data displayed are averages over the 4 writing days for all subjects in the group

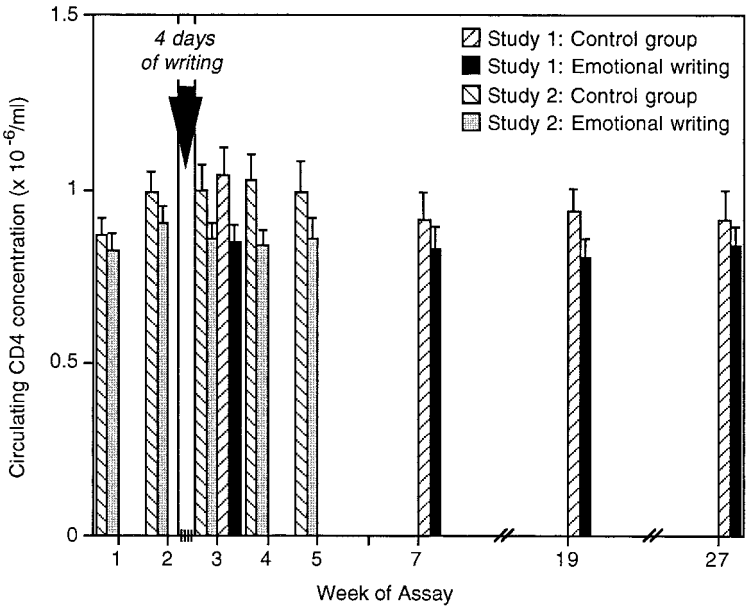

Fig. 2 — Numbers of circulating CD4 lymphocytes in control and emotional writing groups (group mean + SE) over the course of the two studies. The writing intervention occurred over the 4 days before the first blood sample in study 1 and before the third blood sample in study 2

Blood markers

In our paper based on data from study 1,[4] we reported a significant difference in circulating CD4 (helper) lymphocyte numbers between emotional disclosure and control groups the day following the 4 days of writing. A similar effect was observed in study 2 (Fig. 2), with the control group exhibiting higher numbers of circulating CD4 cells after the writing intervention than did the emotional disclosure group. Analysis of variance of combined data from both studies ($N = 78$) using data from blood taken the day after the 4-day writing intervention confirmed a significant main effect of condition (emotional disclosure or trivial writing) ($F(1, 76) = 7.3$, $p < 0.01$) and revealed an additional main effect of sex $F(1, 76) = 8.8$, $p < 0.01$), with females having higher CD4 numbers than males, but no effect of study number nor any interactions among condition, sex and study.

In view of the difference between CD4 numbers in males and females, it was important to determine whether there were preintervention baseline differences between subjects in the writing conditions which could account for the observed postwriting effect. In study 2, two blood samples were donated by subjects in the 2 weeks prior to the writing intervention. There were no significant differences between these preintervention samples, neither by time nor writing condition. Although there were no prewriting blood samples in study 1, subjects in this study donated a final blood sample 6 months after the intervention and at this time there were similarly no significant differences between the two writing groups (Fig. 3). Moreover, there were no significant differences between the 6-month blood data from study 1 and the initial prewriting data from study 2 (see Fig. 3), indicating that these time-points could be legitimately used as 'baseline' values in order to test postwriting variables from a combined dataset (study 1 and study 2). A repeated measures ANOVA model to examine the effects of time (two levels: 'baseline' and immediate postwriting), condition (two levels), sex (two levels) and study number (two levels) showed a significant condition by time effect ($F(1, 71) = 5.3$, $p = 0.02$) but no sex or study number effects and no interaction between these factors and condition.

In order to determine whether the postintervention differences between the two writing groups of subjects were restricted to CD4 numbers, similar repeated measures ANOVAs were carried out on

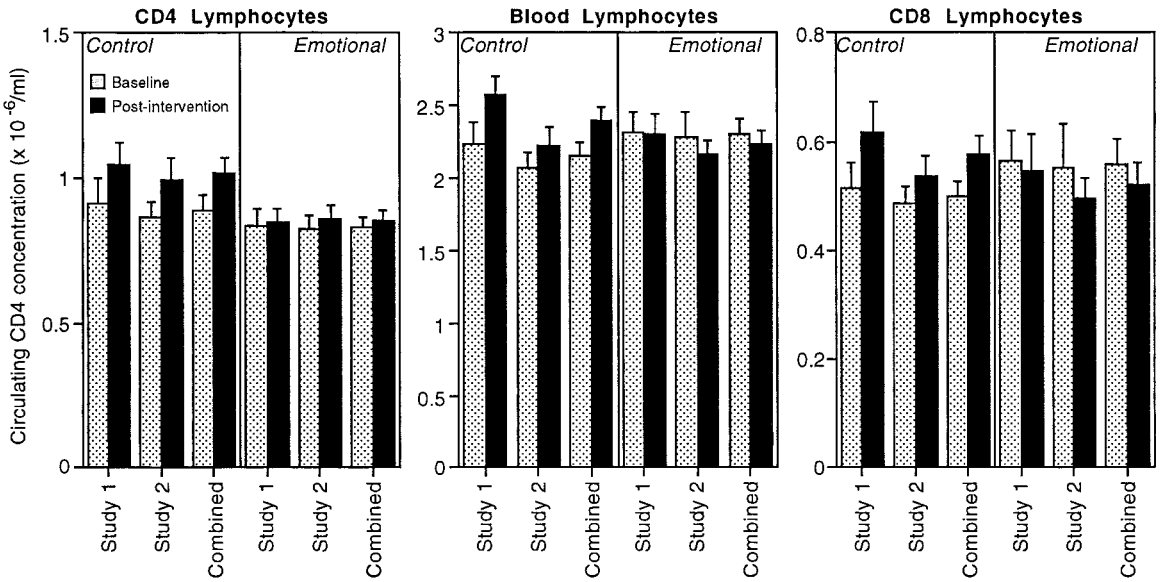

Fig. 3 — Comparisons of CD4, CD8 and total circulating lymphocytes in control and emotional writing groups (group mean + SE) over the course of two studies. Shaded bars indicate baseline values (study 1: initial blood sample; study 2: 6-month blood sample) and black bars represent immediate postintervention values (study 1: initial blood sample; study 2, third blood sample)

other blood cell markers (CD8 numbers, total lymphocyte numbers, CD4 and CD8 percentages, neutrophil numbers, erythrocytes and platelets). Of these, only CD8 numbers and total lymphocyte numbers displayed similar patterns to CD4 numbers (see Fig. 3), with significant postwriting condition by time interactions in CD8 numbers ($F(1,71) = 6.7,\ p = 0.01$) and total lymphocyte numbers ($F(1,71) = 8.6,\ p < 0.01$). None of the other blood markers displayed any intervention-associated changes over time. Further, none of the psychological measures taken before (life orientation, sense of coherence, positive and negative affect, physical symptom inventory) or during (subject self-ratings — see Fig. 1) the writing intervention exhibited any correlation with postwriting CD4 numbers or baseline to postwriting changes in circulating CD4 numbers, CD8 numbers or total lymphocyte numbers within or across writing groups.

DISCUSSION

In this article we report on the effects of emotional disclosure on changes in blood lymphocyte populations over time. The data show that small but reproducible differences in circulating CD4 lymphocyte numbers following a writing intervention were evident in two separate studies. The effect was not specific to the CD4 population, as a similar effect was seen in circulating CD8 numbers and there was no change in CD4/CD8 ratio. Rather, the results indicate a general postwriting increase in the number of circulating lymphocytes within control writing group subjects relative to those in the emotional disclosure group. Notably, the effect was only observed in the circulating lymphocyte population. No group or time differences were evident in other blood cell populations (most importantly neutrophils (phagocytic cells), erythrocytes or platelets).

Time course data (Fig. 2) indicate that the postwriting differences between the two writing groups were most marked in the period immediately following the writing intervention. Further, the emotional writing group exhibited little change in circulating CD4 (CD8 and total lymphocyte) numbers over time, and thus the postwriting differences between the two groups could be attributed to a corresponding rise in these variables in the control group. The differences between the two writing groups could not be accounted for by sex nor by any personality, anxiety-related or

symptom-related measures assessed before the intervention. Two related issues arise from these results.

The first question is how much these results are related to the effect of short-term stress of the writing intervention. Subject self-ratings immediately before and after each writing session demonstrated consistent changes in mood and physical symptom experiences associated with the intervention (Fig. 1). They clearly indicate that subjects in the emotional disclosure group perceived the intervention to be a stressful experience while those in the control group remained relatively unaffected by it. Various short-term immune changes in blood have been reported to be associated with experimental or environmental stresses. These include reduction in lymphocyte mitogenesis,[20–23] changes in helper/suppressor T lymphocyte ratios[20,24,25] and elevated natural killer cell numbers or activity.[20,21,25,26] However, in our studies the subjects who perceived the intervention to be upsetting or stressful (ie the emotional disclosure group) were those whose circulating immune variables displayed little temporal change. One possibility is that any 'stress-related' changes in the emotional disclosure group were relatively short term, lasting less than 24 hours. Alternatively, any short-term effects resulting from the 'stressful' aspects of the emotional writing may have been overridden by its beneficial effects. Recent work from various laboratories including our own supports the latter possibility by demonstrating that emotional disclosure appears positively to influence such immune variables as blastogenic responsiveness to T cell mitogens,[5] response to latent Epstein-Barr virus[7] and antibody generation against hepatitis viral antigens.[4]

A related issue concerns the question of why the number of circulating lymphocyte numbers should rise in the blood of subjects in the control group. Blood lymphocyte populations represent only a few per cent of the total lymphocyte pool and their release into the circulation or sequestration from the circulation by other lymphoid compartments can be quite rapid and affected by a variety of physiological circumstances.[27,28] Given that both these studies were conducted over the winter months, it is conceivable that the temporal variation in circulating lymphocyte populations within the control group was reflective of seasonal and work-related fluctuations within the general student population and that the more consistent pattern observed in the emotional disclosure group over time was a consequence of a 'buffering' effect of the intervention. Zakowski has suggested that predictability may buffer the effect of stressors on immune function.[23] We have previously hypothesized that there is a 'harmony of purpose' relating immune and psychological behaviour[14,15] and as a consequence, integrating or making sense of emotional experiences by constructing a more coherent framework through the disclosure process might have an effect of stabilizing fluctuations in immune variables over time. A trend in our results suggests that those subjects with greater increases in CD4 numbers after writing also experienced more physical symptoms (data not shown), which is in accord with this 'buffering' notion.

Aside from emphasizing that the effects observed do not appear to be explicable as short-term stress reactivity, it is difficult to speculate too much about the clinical significance of a small, statistically significant difference in circulating lymphocyte numbers. It should also be borne in mind that this study was conducted using healthy volunteers and that increasing an immune variable (such circulating lymphocyte numbers) beyond its optimum may not be an indication of a healthy effect. However, in our previous study, stable circulating lymphocyte numbers in the emotional disclosure group were found to correspond with a better response to hepatitis B vaccination.[4] Thus, stability of circulating lymphocyte numbers over time in this context may well be indicative of relative immunological health. Such a possibility could be explored further by including a non-intervention control group for comparison in future studies.

In conclusion, it is clear that the manner in which people handle emotional issues in their lives can affect their health and their immune responsiveness.[4–8] Our results indicate that, following an emotional disclosure writing intervention, circulating lymphocyte numbers were elevated in the control group rather than depressed in the emotional disclosure group and that the only measured variable that was associated with this difference was the intervention condition. Further, although the writing intervention appeared to be a short-term stressor for those in the emotional disclosure group, the subsequent immune effects were not concordant with those associated with short-term stressors in other studies, suggesting that other factors related to the disclosure process itself may be more important here.

ACKNOWLEDGEMENT

This work was supported by grants from the Auckland Medical Research Foundation and the Health Research Council of New Zealand.

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