Effect of stress on immunity: a study among healthy blood donors at King Abdul Aziz University Hospital, Jeddah

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Acute psychological stress is known to alter components of cellular immunity in human beings.[1] For instance, T-suppressor/cytotoxic (CD8) and natural killer (NK) cells in the peripheral circulation typically increase in concentration within minutes of the onset of common stressors.[2] These changes are often attributed to processes such as lymphocyte migration from lymphoid organs to circulating blood or demargination of endothelium-adherent lymphocytes.[3] It is also possible that the lymphocyte population may rise in their relative blood concentration due to a concomitant reduction in plasma volume.[4]

It is becoming more apparent that an understanding of immunocompetence is inadequate in explaining the effect of stress on immunity without consideration of psychological stressors and autonomic psychophysiology. Investigations of these interactions have been stimulated by research demonstrating the direct and moderating effects of psychosocial factors on immune competence.[5] Psychological stress may affect many aspects of the integrative network between the immune, central nervous and endocrine system in both animal and humans. The complex effects of psychological stress on the interactions among these three systems has been subject to studies in the rapidly developing field of psychoneuro-immunology.[6]

Like many medical and dental procedures, blood donation can be a stressful experience,[7] especially for first-time donors.[5] chronic or long-term psychological stressors such as marital strife and bereavement are associated with immunological down-regulation.[8] Unlike these chronic stressors, transient psychological stress is a ubiquitous part of nearly everyone's daily life. The immunological consequences of brief psychological stressors have only recently been examined. Laboratory stressors such as public speaking, academic examination and blood donation provide a model for transient life stressors; these studies of brief psychological stressors have revealed changes in the numbers of circulating mononuclear cells as cell function.[1]

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The prospect of blood donation could be identified as a stressor and the resulting emotional and physiological state could be described as mild stress. This study was designed to analyze the physiologic response to evoked psychological stress (blood donation). The aim was to evaluate the effect of blood donation as a form of mild stress on some aspects of cellular immunity by using flow cytometry in 30 normal, healthy volunteer donors at King Abdulaziz University Hospital (KAAUH) in Jeddah, Saudi Arabia. We also investigated serum immunoglobulin response to acute psychological stress (blood donation).

Methods

Following local ethical committee approval, 30 male donors (aged 20-35 years) were selected under supervision of a medical consultant. Informed consent was obtained from each subject. All subjects were first time donors, in good health, within 20% of their ideal body weight, with no past or present history of chronic illness or psychiatric disease. No subject was taking medical drugs that could interfere with blood donation or immunity, had experienced any recent negative life event (e.g. death in the family), had any occurrence of new medical illnesses, including influenza, alcohol, or heavy smoking, or had a history of fainting related to blood drawing.

Blood samples were taken just before blood donation (pre-stress). For comparison, another sample was taken from the same subject immediately after blood donation (post-stress). Collection was carried out early in the morning. The same amount of blood was taken from each donor and the same procedure for sample collection was applied to each donor to minimize the source of pre-analytical variation. Seven milliliters of venous blood drawn was drawn, 4 mL (in plain tubes) for immunoglobulin analysis and 3 mL (in EDTA tube) for the CBC. The first blood sample (baseline) was drawn to analyze immunoglobulin and lymphocyte subpopulations ($CD4^+$, $CD3^+$, $CD8^+$, $CD14^+$, $CD45^+$, $CD19^+$ and $CD56^+$). A CBC was performed on all samples. Immediately after the end of the blood donation subjects were seated and a second set of blood samples were drawn.

Lymphocytes were stained with a combination of labeled monoclonal antibody and measured by flow cytometry. Expression of surface antigens on gated lymphocytes was assessed by flow cytometry. The absolute

number of circulating lymphocyte was calculated from the result of standard white blood cell counts while the differential analysis was calculated using blood counter machine cell dye 3700.[9] T-cell subsets, B-lymphocytes and natural killer cells percentages and numbers were computed using the Behring ELISA Immunoglobulin Kit (Dade Behring, Liederbach, Germany). Immunoglobulins levels were evaluated before and after blood donation.

Results

CD4 cells decreased while CD8 cells increased significantly, resulting in a lower CD4/CD8 ratio from pre- to post-stress measurements in healthy subjects (Table 1). We also found stress-induced increases of $CD56^+$ cells (NK cells). The significant increase in CD3 cells was representative of the overall increase in the T-cell population. The results suggest a marked stress-induced and long-lasting alteration of T lymphocyte subpopulations after stress. Immunoglobulin levels also changed significantly (Table 2). The mean values for IgG, IgA and, IgM levels increased after blood donation. Serum immunoglobulin levels taken immediately after the blood donation were significantly higher compared with levels before donation (Table 2). The increases of serum immunoglobulins induced by blood donation were comparable to the increases for CD8 and CD56. Changes in lymphocytes and immunoglobulins were within the normal ranges for these immune factors.

Discussion

Our study measured the stress on the immune system induced by the donation of 500 mL of blood. The decrease of $CD4^+$ cells (T helper) and the increase in $CD8^+$ cells (T suppressor) resulted in an imbalance, leading to an overall decrease in the CD4/CD8 ratio. The CD8 lymphocyte subpopulation also has suppressive activity, which may have contributed to the decrease in CD4 cells.[10] The reduction in total blood volume may also have contributed to the imbalance in lymphocyte subpopulations. The decrease in the T helper cells ($CD4^+$) populations, the increase in the percentage of $CD8^+$ cells relative to CD4 and the increase in absolute numbers of T cells are in agreement with previous studies.[11,12] The increase in total T cells, which are represented by the CD3 surface marker, is also in agreement with previously published data.[12] $CD3^+$ cells can be either T helper or T suppressor cells, but there are a small percentage that are non-$CD4^+$ or $CD8^+$. The CD3 surface marker may serve as a control measure for assays of immune system measurements; CD3 is approximately equal to CD4 + CD8.[13-15]

Our data also showed an increase in $CD56^+$ cells (NK), which may suggest an improvement in the immune system. This could be the result of a psychologically positive effect counteracting the stress. The stress induced by the blood donation also resulted in a significant increase (P= 0.0001) of IgG, IgA, and IgM. Increased adrenaline levels may have triggered this increase in immunoglobulins as a means of preparing the body defense system to combat foreign invaders such as bacteria, which may attack under stressful circumstances.

In summary, blood donation, an example of mild stress, may be accompanied by an alteration of T-lymphocyte number and serum immunoglobulin levels. If interactions among the autonomic nervous system, endocrine system, and immune system were amenable to psychophysiological analysis, the underlying mechanisms might be illuminated. Further study is needed to evaluate the long-term effect of mild stress on the numbers and functions of T cells.

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Table 1. Effect of blood donation on lymphocytes.

	Pre-stress percentage (Mean±SD)	Post-stress percentage (Mean±SD)	*P* values
CD4	38.3 ± 6.4	34.7 ± 4.4	0.0001
CD8	29.3 ± 9.1	32.9 ± 8.3	0.0001
CD4/8	1.4 ± 0.5	1.1 ± 0.3	0.0001
CD3	66.2 ± 7.1	68.1 ± 5.2	0.016
CD56	6.4 ± 2.0	9.9 ± 3.9	0.0001

Only variables with statistically significant differences are reported.

Table 2. Effect of blood donation on immunoglobulins.

	Pre-stress (g/L) (Mean±SD)	Post-stress (g/L) (Mean±SD)	*P* values
IgG	13 ± 2.7	13.7 ± 2.8	0.0001
IgA	2.1 ± 0.8	2.3 ± 0.8	0.0001
IgM	1.1 ± 0.5	1.3 ± 0.5	0.0001

References

1. Bachen EA, Manuck SB, Marsland AL, et al. Lymphocyte subset and cellular immune responses to a brief experimental stressor. **Psychosom Med.** 1992; 54:673-679.
2. Naliboff B, Benton D, Solomon G, Morley J, Fahey J, Bloom E, Makinodan T, Gilmore S. Immunological changes in young and old adults during brief laboratory stress. **Psychosom Med.** 1991; 53: 121- 132.
3. Herbert T, Cohen B. Analysis of lymphocyte proliferation data: Do different approaches yield the same results? **Brain Behav Immun.** 1994;8: 153-162.
4. Van Tist LJH, Michel Mc, Grosse- Wilde H, et al. Catecholamines increase lymphocyte B2-adrenergic receptors via a B2-adrenergic, spleen-dependent process. **Am J Physiol.** 1990; 258:EI91-202.
5. Kennedy S, Glaser R, Kiecolt-Glaser J. Psychoneuro-immunology. In: Cacioppo JT, Tassinary LG, eds. **Principles of psychophysiology: Physical, social, and inferential elements.** New York: Cambridge University Press; 1990; 177-190.
6. Sogutas-Emch SA, et al. The effects of, an acute psychological stressor on cardiovascular, endocrine and, cellular immune response. **Psycho-physiology.** 1994; 31: 177 -190.
7. Patterson M, Matthews K, Owens F. Stress- induced hemoconcentration of blood cells and lipids in healthy women during acute psychological stress. **Health Psychol.** 1995;14:319-324.
8. Schleifer J., Keller E. Camerino M., Thomton C.& Stein M. Suppression of lymphocyte stimulation following bereavement. **JAMA.** 1983;250: 374- 377.
9. Keenth D. Bauer E. Duque T. Vincent Shaakey. **Clinical Flow-Cytometry: principles and application.** 1st Ed. Williams & Wilkins, Baltimore, Maryland: USA, 1993: 63-70.
10. De Panfilis G. CD8+ cytolytic T lymphocytes and the skin. **Exp Dermatol.** 1998;7: 121-131.
11. Landmann M, Muller B, Perini H, Wesp M, Erne P, & Booler R. Changes in immunoregulatory cells induced by psychological and physical stress: Relationship to plasma catecholamines. **Clin Exp Immunol.** 1984;58: 127-135.
12. Biselli R, Farrace S, Fattorossi A. Influence of stress on lymphocyte subset distribution-A flow cytometric study in young student pilots. A viat. **Space Environ Med.** 1993;665:116-120.
13. Maes M, Hendriks D, Gastel A, Demedts P, Wauters A, Needs H, Janea A, Scharpe S. Effects of Psychological Stress on Serum Immunoglobulin, complement and Acute Phase Protein Concentrations In Normal Volunteers. **Psychoneuro-endocrinology.** 1997;22(6): 397- 409.
14. Khanna R. Mood and willingness to donate. **Indian J Curr Psychol Res.**1990; 5:109-113.
15. Marsland AL, Tracy B, et al. Lymphocyte subset redistribution during acute laboratory stress in young adults: mediating effects of hemoconcentration. **Am Psychol Assoc Inc.** 1997; 97: 278-6133.