American Journal of Infectious Diseases 3 (1): 17-23, 2007
ISSN 1553-6203

Circulating Granzymes are associated with Bloodstream Infection and Death in Febrile Medical Patients

[1]Annelies N. Tacx, [2]Angela M. Wolbink, [2, 3]C. Erik Hack and [1]A.B. Johan Groeneveld
[1]Department of Intensive Care, [3]Clinical Chemistry and [1,3]Institute for Cardiovascular Research
Vrije Universiteit Medical Center, Amsterdam and [2]Department of Immunopathology
Sanquin Research Amsterdam, The Netherlands

Abstract: Plasma levels of granzymes are specific markers for the activation of cytotoxic T lymphocytes and natural killer (NK) cells but the impact of activation of these cells on the course of bacterial infections is not well understood. We prospectively studied the implications of circulating granzyme A and B (GrA, GrB) levels in febrile, medical and hospitalized patients (n=284), during 3 days after inclusion and related levels to lymphocyte counts, infection, sepsis, culture results, shock and infection-related 28-day mortality. Bloodstream infection occurred in 18% of the patients, 9% died within 28 days. Circulating GrA and GrB levels were elevated, at inclusion, in 98 and 55% of patients. The course of GrB predicted bloodstream infection whereas that of GrA predicted mortality, regardless of relative lymphocytopenia. Our data suggest harmful cytotoxic T and NK cell activation early in bacterial infection-related fever. They also point to an early but different pathogenic role of soluble GrA and GrB when fever is associated with mortality and bloodstream infection, respectively.

Key words: Granzymes, sepsis, apoptosis, prognosis, fever in hospital

INTRODUCTION

Granzymes A and B (GrA, GrB) are serine proteases that mediate apoptosis when released by activated cytotoxic T-lymphocytes and natural killer (NK) cells into target cells[1-5]. The enzymes are involved in the defense against viral infections, allogenic donor tissues and tumors[1-5]. GrA and B are also released into interstitial fluid and plasma upon activation and their levels in biological fluids can be used as an index of activation of cytotoxic T and NK cells[1, 5]. Whether extracellular granzymes exert biological effects is not clear, although it has been suggested that they may be involved in degradation of extracellular matrix molecules, among others[1, 5]. In any case, NK cell activation contributes to harmful effects of endotoxin in experimental animals[6]. Hence, elevated levels of granzymes in the blood during human endotoxemia and established sepsis, suggests a role for cytotoxic T and NK cells in the defense mechanisms against bacteria[7-9]. Granzyme release could contribute to cytokines release, multiple organ failure and death during sepsis, which are believed to result in part from apoptosis[9-12]. However, the precise pathogenic role of circulating granzymes and its timing in serious non-viral infections in man are still unclear[9]. Indeed, release prior to adverse sequelae of infection would favor a mediator rather than a marker role. Moreover, advanced sepsis and bacteremia are characterized by lymphocytopenia, probably as a consequence of lymphocyte apoptosis and thereby perhaps contributing to persistent or recurrent sepsis and to death[10, 13-16]. Hence, lymphocyte activation and granzyme release may be associated with lymphocyte apoptosis and lymphocytopenia[3, 13, 16-18].

To get more insight into the activation of cytotoxic T and NK cells in the early phase of sepsis, we measured serially GrA and GrB plasma levels in hospitalized medical patients with newly onset fever and related levels to infection, sepsis, culture results, shock, lymphocytes, IL-6 and infection-related outcome.

Patients and methods: The patients included in this study participated in a protocol in which 300 consecutive patients with newly onset fever (body

Corresponding Author: A.B. Johan Groeneveld, M.D., Ph.D., F.C.C.P., F.C.C.M., Intensive Care, V.U. University Medical Center, De Boelelaan 1117, 1081 HV Amsterdam, The Netherlands

temperature >38 °C axillary or 38.3 °C rectally), admitted to the Department of Internal medicine in an University Hospital, were included, as described elsewhere[19]. For the present study plasma samples from 284 patients were available. The study was approved by the local committee on ethics. All patients or their closest relatives gave informed consent before inclusion. Exclusion criteria were pregnancy, use of cytokines such as interferon-gamma or interleukin (IL)-2, treatment for malignant hematological disease, shock and a life expectancy of less than 24 hours. Patients were taken care for by attending physicians not involved in the study.

At inclusion, patient characteristics were recorded. Clinically presumed foci of infection, as judged by treating physicians were recorded. Presumed sepsis was defined when patients met criteria for the systemic inflammatory response syndrome in the presence of a clinical infection and microbiologically proven sepsis when accompanied by positive local and/or blood cultures for pathogens.

The development of shock was evaluated during a maximum follow up of 7 days if patients were still in hospital. Shock was defined by hypotension (systolic arterial blood pressure <90 mm Hg or a reduction >40 mm Hg from baseline values), in the absence of other causes for hypotension and despite adequate fluid resuscitation or by the need for administering vasoactive drugs to increase the pressure. Outcome in the hospital was assessed during a follow-up for a maximum of 28 days after inclusion. Patients discharged from the hospital in the follow-up period were classified as survivors. Five patients died of disease thought to be unrelated to fever and were therefore considered as having survived the febrile episode. They were considered as survivors since the temperature had returned to normal and in the opinion of the treating physician, the infectious focus had been cured before death. Causes of death in these patients were apnea due to aspiration, cardiac arrest in a patient with metastatic breast cancer, metastatic lung cancer, cardiogenic shock due to cardiomyopathy and respiratory insufficiency due to cervical cord dissection.

Microbial cultures: At least two venous blood samples were obtained at inclusion for microbiological studies. Supplementary blood cultures were taken when clinically indicated. Results from microbiological studies performed during the first seven days after inclusion, were recorded. Patients were classified into 2 groups with and without positive cultures. Blood cultures were processed using "delayed vial entry" bottles for aerobic and anaerobic cultures and the Bactec 9120/9240 automatic analyzers (Becton Dickinson, Erembodegem, Belgium). Bottles were incubated for a maximum of seven days. If the analyzers showed growth, Gram stains were prepared and sensitivity of the organism for antibiotics was assessed. Blood cultures containing *S. epidermidis* were considered contaminated if only one bottle revealed growth and there were no indwelling vascular catheters. Local cultures were taken at the discretion of the treating physician.

Measurements: Leukocyte counts normally vary between 4-12 $x10^9$ L^{-1} (Sysmec SE 9000 analyzer, Kobe, Japan). Differential counts were done and numbers of lymphocytes were expressed in absolute numbers and as percentage of leukocyte counts (normal >1.0 $x10^9$ L^{-1} and 15-40% of total leukocytes). At inclusion blood was taken for determination of plasma IL-6 with a modified ELISA[20]. Normal values in healthy volunteers are less than 10 pg mL^{-1}. Blood samples for determination of plasma levels of granzymes were obtained at inclusion and in the morning on the two following days. The samples were collected in tubes containing soybean trypsin inhibitor (100 $microg.mL^{-1}$, final concentration), ethylene-diaminetetraacetate (EDTA, 10 $mmol.L^{-1}$) and benzamidin (10 $mmol.L^{-1}$), to prevent in vitro activation. All tubes were centrifuged for 10 minutes at 1,300 g and aliquots of the plasma were stored immediately at -70 °C until assays were performed. Plasma levels of granzymes were measured with specific ELISAs[1, 7, 21]. Detection limits, upper level of normal and mean level in healthy volunteers are, for GrA 11, <60 and 21 $pg.mL^{-1}$ and for GrB 6, <40 and 12 $pg.mL^{-1}$, respectively[1, 7, 21].

Statistical analysis: Groups were compared using Mann-Whitney U tests. Multiple logistic regressison (backward method on the basis of likelihood ratio) was used to find the smallest set of independent predictors of 28-day mortality. The Hosmer-Lemeshow test was used to judge the model fit. Receiver operating characteristic curves, plotting sensitivity against 1-specificity, were made for prediction of bloodstream infection and death, according to granzyme levels. The more the area under the curve approaches 1 the better the predictive value. Spearman rank correlation coefficients were used. A two-sided $P<0.05$ was considered statistically significant; exact P values are given unless <0.001. Values are summarized by median (range) except for graphical presentation (mean±standard error of the mean).

RESULTS

Table 1 describes patient demographics, underlying diseases, infection, sepsis, culture results, shock and outcome. Of the 284 patients, 52 (18%) had bloodstream infection. There were 25 deaths. Bloodstream infection predisposed to shock (P<0.001) and shock to death (P<0.001), since 9 of 15 shock patients had bloodstream infection and died. Table 2 shows some inflammatory markers at inclusion. There was often relative lymphocytopenia, but 164 (89%) of patients had absolute lymphocyte counts above the normal of 1.0 x10^9 L^{-1} at day 0.

Table 1: Patient characteristics (n=284)

Gender, male/female	145 (51)/139 (49)
Age, year	63 (17-70)
Underlying disease	
Cancer	64 (23)
Cardiovascular	61 (21)
Urogenital	44 (15)
Diabetes mellitus	42 (15)
Recent surgery	33 (12)
Respiratory	30 (11)
Autoimmune disease	27 (10)
Neurological	26 (9)
Coming from home	247 (87)
Community-acquired fever	207 (73)
Presumed clinical focus of infection	199 (70)
Presumed sepsis	190 (67)
Microbiologically proven sepsis	120 (42)
Positive cultures	127 (45)
Bacteria in blood culture	
Enterobacteriaceae	18 (6)
Staphylococcus spp.	15 (5)
Streptococcus spp.	9 (3)
Anaerobic Gram-negative	4 (1)
Pseudomonas spp.	2 (1)
Miscellaneous	4 (1)
Shock	15 (5)
Mortality	25 (9)

Median/range or number (percentage) of patients, where appropriate.

Circulating granzymes: At day 0, GrA and GrB levels were elevated above the detection limit in 240 and 135 of the 244 patients tested, respectively. The number of patients and determinations declined in time because of discharge or death.

Infection and sepsis: Patients with a presumed clinical focus of infection had greater relative lymphopenia (P=0.005) and higher GrA at day 0 than patients without a focus (Table 3). Patients with positive cultures had higher GrB levels on day 1 than those without, whereas highest levels in the course of time

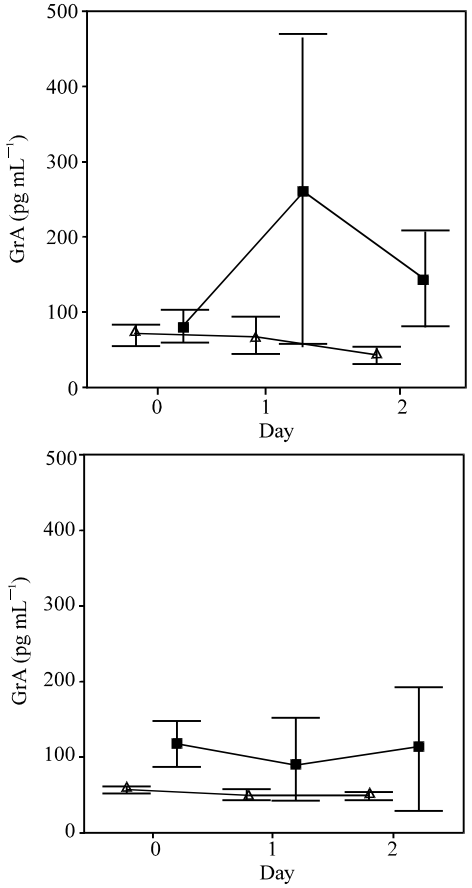

Fig. 1: A: Course of granzyme (Gr) A levels in patients with bacteremia. B: without bacteremia. Triangles: non-survivors, squares: survivors. P=0.002 for day 2 in bacteremia (A) and P=0.001 for day 0 without bacteremia (B). Numbers refer to number of patients. Mean±SEM

also differed (P=0.024). Patients with microbiologically proven sepsis had higher GrB levels on day 1 than those without, whereas highest GrB levels also differed (P=0.050).

Bacteremia and shock: The GrB levels were higher in patients with than without bloodstream infection on day 0 and 1. Ratios of levels of GrA to GrB were therefore lower in patients with than without bloodstream infection (P<0.001 day 0 to 0.020 day 2). GrB was elevated in patients with bloodstream infection versus those without, regardless of outcome (day 0 and 1, P=0.001 and 0.027, respectively, for survivors and P=0.04 for day 0 in non-survivors), while GrA did not differ. The highest GrB level in the course of time was

Table 2: Indicators of inflammation (day 0)

	Bacteremia n=52	No bacteremia n=232	Survival n=259	Death n=25
Temperature, °C	39.1 (38.4-40.5)	38.9 (38.0-41.3)[3]	38.9 (38.0-41.3)	39.0(38.3-39.9)
Leukocytes, $x10^9$/L	12 (1-38)	11 (0.3-156)	11 (0.3-42)	15 (3-156)
Lymphocytes, $x10^9$/L	1.0 (0-2.2)	0.9 (0.01-4.1)	0.9 (0-4.1)	1.0 (0.5-2.8)
Lymphocytes, %	7 (0-20)	10 (1-80)	10 (0-80)	13 (3-31)
IL-6, pg/Ml	72 (4-10000)	25 (1-3502)[1]	26 (1-10000)	93 (6-3502)[2]

Median (range). BSI, bloodstream infection. [1]P<0.001; [2]P=0.010; [3]P=0.038

Table 3: Granzyme levels (pg/mL)

	Granzyme A			Granzyme B		
	Yes	No	P	Yes	No	P
Presumed clinical focus of infection, n=199						
day 0	43 (1-297)	52 (1-521)	0.022	9 (1-1541)	9 (1-441)	
day 1	47 (1-286)	48 (11-1497)		10 (1-1051)	10 (1-989)	
day 2	43 (5-172)	37 (6-341)		9 (1-1103)	10 (1-717)	
Microbiologically proven sepsis, n=120						
day 0	49 (1-521)	44 (1-341)		12 (1-515)	6 (1-1541)	
day 1	42 (2-1497)	48 (1-217)		11 (1-989)	8 (1-1051)	0.022
day 2	42 (5-341)	39 (6-195)		10 (1-717)	9 (1-1103)	
Positive culture, n=127						
day 0	49 (1-521)	44 (1-341)		12 (1-515)	6 (1-1541)	
day 1	42 (2-1497)	48 (1-217)		11 (1-989)	8 (1-1051)	0.022
day 2	43 (5-341)	39 (6-195)		9 (1-717)	9 (1-1103)	
Bacteremia, n=52						
day 0	50 (11-339)	44 (1-521)		24 (1-515)	6 (1-1541)	<0.001
day 1	50 (5-1497)	45 (1-286)		18 (1-989)	9 (1-1051)	0.005
day 2	42 (5-341)	39 (6-195)		10 (1-717)	9 (1-1103)	
Shock, n=15						
day 0	54 (17-132)	46 (1-521)		21 (3-515)	8 (1-1541)	
day 1	54 (20-286)	45 (1-1497)		19 (3-385)	10 (1-1051)	
day 2	57 (7-122)	40 (5-341)		16 (1-383)	9 (1-1103)	
Non-survival, n=25						
day 0	81 (11-521)	44 (1-341)	<0.001	18 (1-319)	8 (1-1541)	
day 1	82 (14-1497)	46 (1-594)		25 (5-832)	10 (1-1051)	
day 2	92 (29-341)	38 (5-283)	0.01	22 (8-717)	9 (1-1103)	

Median (range).

higher in patients with bloodstream infection (P<0.001) and in patients developing shock (P=0.008 or lower) than in those without.

Non-survivors versus survivors : GrA levels were higher in non-survivors than in survivors. In patients with bloodstream infection, GrA and GrB levels at day 2 were higher in non-survivors than in survivors (P=0.002 and 0.026, respectively). In patients without bloodstream infection, only the GrA level on day 0 was higher in non-survivors (P=0.001). The highest GrA level was also elevated in non-survivors (P=0.003). The Fig. 1-2 show the time course of GrA and GrB levels according to presence of bloodstream infection and to survival.

Correlations: GrA related to GrB on each day (maximum r_s=0.54, P<0.001). At day 0, GrA levels somewhat directly correlated with circulating absolute and relative lymphocyte counts (minimum r_s=0.19, P=0.017), which related inversely to IL-6 levels (r_s=-0.32, P<0.001). IL-6 levels did not relate to those of granzymes and there was no difference in granzyme release in Gram-positive versus Gram-negative bloodstream infections.

Prediction: In multiple logistic regression, the highest GrA level (P=0.014), the presence of cancer (P=0.019) and shock (P<0.001) were predictor of death on day 28, independent of each other and of age, sex, comorbidity, infectious focus, cultures, lymphocytes and IL-6 levels (X^2=5.3, P=0.73). The area under the receiver operating

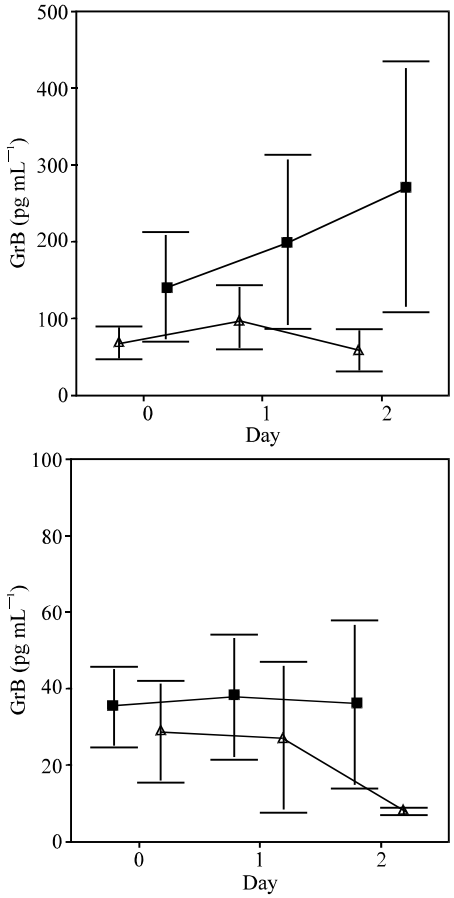

Fig. 2: A:Course of granzyme (Gr) B levels in patients with bacteremia. B: without bacteremia. Triangles: non-survivors, squares: survivors. P=0.026 for day 2 in bacteremia (A). Numbers refer to number of patients. Mean±SEM

characteristic curve for bloodstream infection prediction was greatest for GrB levels on day 1 (0.71, P=0.022). The area for prediction of death was greatest for GrA level on Day 0 (0.91, P=0.007).

DISCUSSION

We show release of pro-apoptotic GrA/B into the circulation in febrile patients, particularly, but differentially, in those having a bloodstream infection and developing an unfavorable course. Moreover, granzyme changes were independent of relative lymphocytopenia. These results support an early pathogenic role of cytotoxic T lymphocyte and NK cell activation in severe bacterial infections, as in experimental models of endotoxin shock[6].

The relative lymphocytopenia found in our study has been described before, in septic animals and man[7, 13-15]. In our study, the absolute or relative numbers of lymphocytes did not relate to bloodstream infection and a poor outcome, in contrast to other reports[14, 15]. It has been suggested that increased apoptosis of lymphocytes contributes to lymphocytopenia and decreased immune responses thereby predisposing to persistent or subsequent infection and mortality, even in human sepsis[10,13,14,16]. Evidence for apoptosis of lymphocytes and other cells in sepsis is accumulating. Indeed, granzymes are released from cytotoxic T lymphocytes or NK cells as a result of activation and activated cells may undergo more rapidly apoptosis than non-activated cells, primarily related to GrB[3,13,16-18]. Alternatively, our data point to another mechanism underlying lymphocytopenia in sepsis, i.e. activation of NK cells and subsequent adhesion to the endothelium and/or sequestration in the micro-circulation leading to increased permeability, among others[22]. Phenotypic analysis of circulating lymphocytes may have further supported the concept of activated T-lymphocytes and NK cells, but we did not do such an analysis in the patients.

Our data confirm and extend studies on human endotoxemia, melioidosis and established sepsis[7-9], which demonstrate that non-viral bloodstream infection is accompanied by activation of cytotoxic T lymphocytes and NK cells at an early stage to release granzymes and that the latter play a pathogenic role. The different behaviour of GrA and GrB regarding outcome and bloodstream infection in our study, needs further explanation since this has not been described before. The lower ratio of GrA to GrB levels during bloodstream infection suggested preferential release of the latter, in accordance with meningococcal disease in children[8] but contrasting with the earlier and greater elevation of GrA than of GrB in blood after injection of endotoxin in human volunteers[7]. However, GrA and GrB are expressed in different lymphocyte subsets and bacterial products in blood may thus have differentially activated subsets in our patients[23]. Overall, GrA levels were of greater prognostic significance than those of GrB, which were reported before to have little prognostic significance in human sepsis[7,9]. Among possible explanations for these differences are different pro-apoptotic pathways and clearance mechanisms of either granzyme[5]. Relevant in this respect may be that in contrast to GrB, circulating GrA may be protected from inactivation by proteases and promote

inflammatory mediator production[1,21,24]. There was no relation between IL-6 and GrA/B levels in our patients, however.

CONCLUSION

Circulating granzyme levels are related to the presence of bloodstream infection and outcome in patients with fever. Our data suggest an early role for granzymes in the pathogenesis of sepsis.

ACKNOWLEDGEMENTS

Anke Eerenberg-Belmer, Ingrid van de Hul and Erna Alberts are gratefully acknowledged for superior technical assistance.

REFERENCES

1. Spaeny-Dekking E.H.A., W.L. Hanna, A.M. Wolbink, P.C. Wever, A.J. Kummer, A.J.G. Swaak, J.M. Middeldorp, H.G. Huisman, C.J. Froelich and C.E. Hack, 1998. Extracellular granzymes A and B in humans: Detection of native species during CTL responses *in vitro* and *in vivo*. J. Immunol., 160: 3610-3616.
2. Trapani, J., 2001. Granzymes: A family of lymphocyte granule serine proteases, Gen. Biol., 3014: 1-7.
3. Regner, M. and A. Mullbacher, 2004. Granzymes in cytolytic lymphocytes - to kill a killer? Immunol. Cell Biol., 82: 161-169.
4. Ashton-Rickardt, P.G., 2005. The granule pathway of programmed cell death. Crit. Rev. Immunol., 25: 161-182.
5. Buzza, M.S. and P.I. Bird, 2006. Extracellular granzymes: Current perspectives. Biol. Chem., 387: 827-837.
6. Emoto, M., M. Miyamoto, I. Yoshizawa, Y. Emoto, U.E. Schaible, E. Kita and S.H.E. Kaufmann, 2002. Critical role of NK cells rather than $V\alpha14^{+}$ NKT cells in lipopolysaccharide-induced lethal shock in mice. J. Immunol., 169: 1426-1432.
7. Lauw, F.N., A.J.W. Simpson, C.E. Hack, J.M. Prins, A.M. Wolbink, S.J.H. Van Deventer, W. Chaowagul, N.J. White and T. Van der Poll, 2000. Soluble granzymes are released during human endotoxemia and in patients with severe infection due to gram-negative bacteria. J. Infect. Dis., 182: 206-213.
8. Van Woensel, J.B.M., M.H. Biezeveld, C.E. Hack, A.P. Bos, T.W. Kuijpers, 2005. Elastase and granzymes during meningococcal disease in children: correlation to disease severity. Inten. Care Med., 31: 1239-1247.
9. Zeerleder, S., C.E. Hack, C. Caliezi, G. van Mierlo, A. Eerenberg-Belmer, A. Wolbink and W.A. Wuillenmin, 2005. Activated cytotoxic T cells and NK cells in severe sepsis and septic shock and their role in multiple organ dysfunction. Clin. Immunol., 116: 158-165.
10. Hotchkiss, R.S., P.E. Swanson, B.D. Freeman, K.W. Tinsley, J.P. Cobb, G.M. Matuschak, T.G. Buchman and I.E. Karl, 1999. Apoptotic cell death in patients with sepsis, shock and multiple organ dysfunction. Crit. Care Med., 27: 1230-1251.
11. Hashimoto, S., A. Kobayashi, K. Kooguchi, Y. Kitamura, H. Onodera and H. Nakajima, 2000. Upregulation of two death pathways of perforin/granzyme and FasL/Fas in septic acute respiratory distress syndrome. Am. J. Respir. Crit. Care Med., 161: 237-243.
12. Zeerleder, S., B. Zwart, W. Wuillemion, L.A. Aarden, A.B.J. Groeneveld, C. Calezi, A.E.M. Van Nieuwenhuijze, G.J. Van Mierlo, A.J.M. Eerenberg, B. Lämmle and C.E. Hack, 2003. Elevated nucleosome levels in systemic inflammation and sepsis. Crit. Care Med., 31: 1947-1951.
13. Krabbe, K.S., H. Bruunsgaard, J. Qvist, L. Fonsmark, C.M. Moller Khansen, P. Skinhoj and B.K. Pedersen, 2002. Activated T lymphocytes disappear from circulation during endotoxemia in humans. Clin. Diagn. Lab. Immunol., 9: 731-735.
14. Le Tulzo, Y., C. Pangault, A. Gacouin, V. Guilloux, O. Tribut, L. Amiot, P. Tattevin, R. Thomas, R. Fauchet and B. Drenou, 2002. Early circulating lymphocyte apoptosis in human septic shock is associated with poor outcome. Shock, 18: 487-494.
15. Wyllie, D.H., I.C.J.W. Bowler and T.E.A. Peto, 2004. Relation between lymphopenia and bacteraemia in UK adults with medical emergencies. J. Clin. Pathol., 57: 950-955.
16. Hotchkiss, R.S., S.B. Osmon, K.C. Chang, T.H. Wagner, C.M. Coopersmith and I.E. Karl, 2005. Accelerated lymphocyte death in sepsis occurs by both the death receptor and mitochondrial pathways. J. Immunol., 174: 5110-5118.
17. Wever, P.C., H.J.J. Van der Vliet, L.H.A. Spaeny,

A.M. Wolbink, F.N.J. Van Diepen, C.J. Froelich, C.E. Hack and I.J.M. Ten Berge, 1998. The CD8+ granzyme B+ T-cell subset in peripheral blood from healthy individuals contains activated and apoptosis-prone cells. Immunology, 93: 383-389.

18. Ida, H., P.J. Utz, P. Anderson and K. Eguchi, 2005. Granzyme B and natural killer (NK) cell death. Mol. Rheumatol., 15: 315-322.
19. Groeneveld, A.B.J., A.W.J. Bossink, G.J. Van Mierlo and C.E. Hack, 2001. Circulating inflammatory mediators in patients with fever: Predicting blood stream infection. Clin. Diag. Lab. Immunol., 8: 1189-1195.
20. Helle, M., E. Boeije, E. de Groot, A. de Vos and L.A. Aarden, 1991. Sensitive Elisa for interleukin-6. Detection of Il-6 in biological fluids: Synovial fluids and sera. J. Immunol. Methods., 138: 47-56.
21. Spaeny-Dekking, E.H.A., A.M. Kamp, C. Froelich and C.E. Hack, 2000. Extracellular granzyme A complexed to proteoglycans, is protected against inactivation by protease inhibitors. Blood, 95: 1465-1472.
22. Damle, N.K. and L.V. Doyle, 1990. Ability of human T lymphocytes to adhere to vascular endothelial cells and to augment endothelial permeability to macromolecules is linked to their state of post-thymic maturation. J. Immmunol., 144: 1233-1240.
23. Grossman, W.J., J.W. Verbsky, B.L. Tollefsen, C. Kemper, J.P. Atkinson and T.J. Ley, 2004. Differential expression of granzymes A and B in human cytotoxic lymphocyte subsets and T regulatory cells. Blood, 104: 2840-2848.
24. Sower, L.E., G.R. Klimpel, W. Hanna and C.J. Froelich, 1996. Extracellular activities of human granzymes. I. Granzyme A induces IL-6 and IL-8 production in fibroblast and epithelial cell lines. Cell. Immunol., 171: 159-163.