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Patient Cytokine Response in Transfusion-Associated Sepsis

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Cytokine concentrations in plasma from patients transfused with packed erythrocytes contaminated with gram-negative bacilli were measured. Cytokine concentrations in posttransfusion plasma were significantly elevated. A difference in cytokine patterns between survivors and a nonsurvivor was observed.

Although transfusion reactions due to bacterially contaminated blood are a rare occurrence, reports of such reactions have increased over the past 8 years (19). Since 1986, 19 episodes of transfusion reaction due to packed erythrocytes (PRBCs) contaminated with gram-negative bacilli have been investigated by the Centers for Disease Control and Prevention (CDC). Eleven (58%) of these episodes resulted in fatalities. In all 19 episodes, the PRBCs were contaminated with gram-negative bacilli capable of growth and endotoxin production at 4°C. *Yersinia enterocolitica* was implicated in 15 episodes, *Serratia liquefaciens* was implicated in 2 episodes, and *Enterobacter agglomerans* and *Janthinobacterium lividum* B were implicated in 1 episode each.

Bacterial endotoxin present in the outer membrane of all gram-negative bacteria is a potent stimulator of macrophages and the complement and coagulation systems (15, 18). Cytokines released from activated macrophages play a key role in the body's response to gram-negative sepsis and endotoxemia. Tumor necrosis factor alpha (TNF-α), interleukin-1 beta (IL-1β), IL-6, and IL-8 are released in a complex cascade and mediate reactions associated with septic shock and death (5, 8, 14, 20).

Our study examined the effects of transfusion-associated sepsis on cytokine concentrations in patient plasma. TNF-α, IL-1β, IL-6, and IL-8 concentrations in patient plasma were measured pre- and posttransfusion.

Thirty plasma samples from eight recipients of contaminated PRBCs were sent to the CDC by overnight mail. Upon receipt at the CDC, the plasma samples were stored frozen at −70°C until the time of assay. Cytokines were measured by using an enzyme-linked immunosorbent assay (ELISA). TNF-α, IL-1β, and IL-6 ELISA kits (Cistron Biotechnology, Pinebrook, N.J.) and IL-8 Quantikine kits (R & D Systems, Minneapolis, Minn.) were used according to manufacturers' recommendations. Although the sensitivities stated by the manufacturers are 18 pg/ml for IL-8 and 20 pg/ml for TNF-α, IL-1β, and IL-6, we have found concentrations as low as 1 pg/ml to give reproducible results (17). As suggested by the manufacturer, standard curves were made from pooled healthy control plasma that was heat inactivated at 56°C for 1 h to inactivate endogenous cytokines. When necessary, samples that exceeded the standard curve range were diluted 1:10 or 1:100 in sterile saline and retested.

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Plasma cytokine concentrations varied according to time after transfusion (Table 1). The levels of all four cytokines measured were significantly elevated 1 to 4 h posttransfusion. TNF-α, IL-1β, and IL-8 peaked 1 to 8 h posttransfusion. However, the median IL-1β concentration during this time period was 3 logs lower than the median concentration of TNF-α or IL-8. IL-6 peaked 9 to 12 h posttransfusion. TNF-α and IL-1β returned to pretransfusion levels 19 to 28 h posttransfusion. IL-6 and IL-8 concentrations decreased by 2 logs within 24 h but remained above pretransfusion levels.

Sequential plasma samples that enabled evaluation of the temporal cytokine response were obtained from three patients (Fig. 1).

Case 1. A 50-year-old female with breast cancer received approximately 150 ml of a 26-day-old PRBC unit. The remainder of the unit was refrigerated and sent to the CDC on ice by overnight mail 5 days after the transfusion reaction. At the CDC, the unit was found to contain 6.5×10^7 CFU of *J. lividum* B per ml and 45.5 ng of endotoxin per ml.

Case 2. A 74-year-old male undergoing total knee replacement received approximately 100 ml of a 41-day-old autologous PRBC unit. The PRBCs were stored and sent to the CDC as described for case 1. The PRBCs were received 14 days after transfusion and were found to contain 2.7×10^8 CFU of *Y. enterocolitica* serotype O:3 per ml and 3,500 ng of endotoxin per ml. *Y. enterocolitica* was also isolated from the patient's blood posttransfusion.

Case 3. An 83-year-old male recovering from portacaval shunt surgery necessitated by liver disease received approximately 35 ml of a 22-day-old PRBC unit. *Y. enterocolitica* serotype O:3 was recovered from the unit and the patient. The unit was not available for bacterial quantitation or endotoxin assay. However, in growth studies, we found that *Y. enterocolitica* reached concentrations of approximately 10^6 CFU/ml in PRBCs within 22 days (1).

In cases 1 and 2, all four cytokines increased rapidly in the first 4 h posttransfusion and then steadily declined. By 24 h, TNF-α and IL-1β returned to pretransfusion levels and IL-6 and IL-8 decreased 1 to 2 logs. In case 3, TNF-α remained elevated and IL-1β showed a slight increase at 22 h. IL-8 decreased by 1 log, but there was no decrease in IL-6 concentration.

The cytokine profiles for cases 1 and 2 are very similar to each other and to the median concentrations in the 30 patient samples (Table 1). Although the relationship between cytokine concentration and patient survival could not be evaluated because of the small number of patients, the cytokine profile for case 3, in which the patient died, was different from that for cases 1 and 2, in which both patients survived. In case 3, TNF-α

TABLE 1. Median cytokine concentrations in plasma from patients with episodes of transfusion-associated sepsis, 1989 to 1992

Time (h) posttransfusion	No. of specimens	Median concn (pg/ml) of cytokine in plasma[a]			
		TNF-α	IL-1β	IL-6	IL-8
0 (pretransfusion)	4	0	0	70 (0–170)	40 (0–260)
1–4	5	104,800 (19,400–157,000)	280 (20–2,200)	483,900 (141,600–938,000)	125,300 (44,900–372,100)
5–8	4	97,100 (59,600–155,400)	40 (0–90)	488,400 (134,200–861,100)	78,500 (32,500–120,600)
9–12	4	5,700 (530–9,700)	110 (0–130)	760,700 (32,200–1,100,000)	37,100 (22,800–146,400)
13–18	3	100 (100–210)	80 (20–110)	318,800 (297,800–344,400)	6,100 (5,800–96,100)
19–28	4	0 (0–70)	0 (0–160)	24,600 (4,000–260,500)	3,700 (170–12,600)
>28	6	10 (0–20)	0	150 (0–1,300)	60 (10–220)

[a] Values in parentheses are ranges.

and IL-1β did not return to baseline and IL-6 concentrations exceeded 1,000 ng/ml and persisted until the patient's death at 22 h. This is similar to what has been reported by Creasey et al., who found that IL-6 concentrations continued to rise in experimental lethal bacteremia (6). Persistent or increasing concentrations of TNF-α and IL-6 in nonsurvivors have been reported in other studies of patients with septic shock (3, 4, 7, 16). In our three case patients, IL-8 followed the pattern of IL-6 closely, as has been reported by Halstensen et al. for meningococcal patients (11). Although the level of IL-1β response to overwhelming bacteremia in our case patients was lower than the levels of the other cytokines measured, a difference was seen in the profile of this cytokine in case 3, in which IL-1β persisted until death of the patient.

Bacterial and endotoxin loads did not seem to correlate with cytokine response or survival in the three case patients, as the estimated bacterial load was lowest for case 3, in which the patient died. Rather, patient outcome and cytokine response are most likely determined by the complex relationship among many factors, including host immune response, severity of illness, and bacterial and endotoxin loads (2, 3, 16).

Concentrations of cytokines in our case patients were extremely high and are comparable to those reported by Waage et al. for patients with overwhelming meningococcal infection (21–23). In contrast to patients in other studies of sepsis, the patients in cases 1 and 2 survived despite the high concentrations of TNF-α and IL-6 (10, 22, 23). Because of the rapid and recognizable onset of symptoms associated with transfusion reactions and the high concentrations of cytokines involved, future research should assess whether anticytokine therapy, if

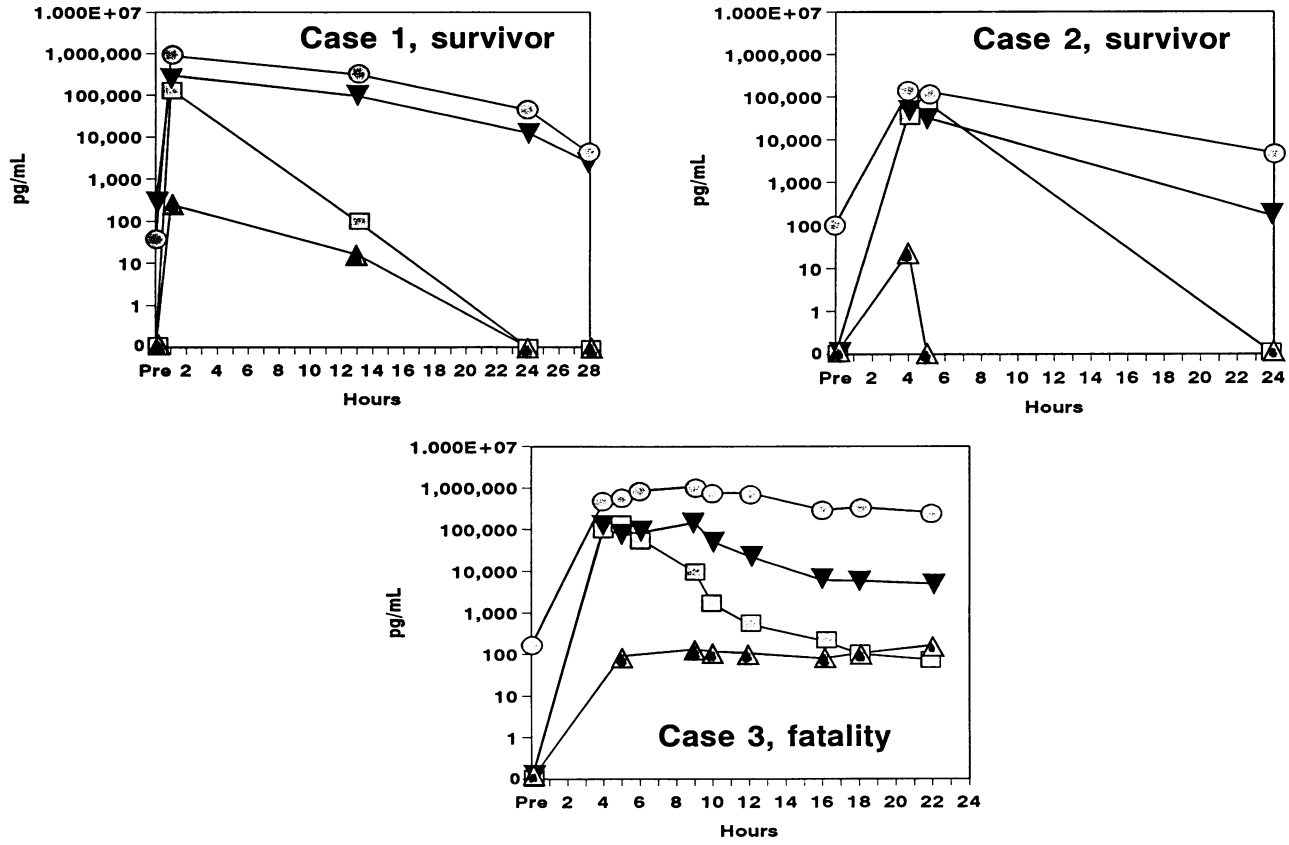

FIG. 1. Concentrations of TNF-α (■); IL-1β (▲); IL-6 (●), and IL-8 (▼) in plasma from three patients with transfusion-associated sepsis.

properly timed, could benefit septic patients who are at high risk of mortality (9, 12).

Overwhelming gram-negative sepsis and endotoxemia leading to septic shock are associated with high mortality rates (2, 13, 24). Cytokines released in response to bacteria and endotoxin are responsible for many of the systemic effects involved in septic shock (5, 8, 14, 20). Transfusion-associated sepsis due to PRBCs contaminated with gram-negative bacilli is a continuing problem, and the fatality rate is high. Bacterial and endotoxin concentrations in PRBCs stored at 4°C can reach very high levels within 2 weeks of collection. As seen in our study, transfusion-associated sepsis is accompanied by very high cytokine concentrations. New therapies for the treatment of septic shock may increase survival; however, continued reports of transfusion-associated sepsis underscore the need for a rapid and efficacious method for screening blood units to detect bacteria or endotoxin.

REFERENCES

1. **Arduino, M. J., L. A. Bland, M. A. Tipple, S. M. Aguero, M. S. Favero, and W. R. Jarvis.** 1989. Growth and endotoxin production of *Yersinia enterocolitica* and *Enterobacter agglomerans* in packed erythrocytes. J. Clin. Microbiol. **27**:1483–1485.
2. **Bone, R. C., C. J. Fisher, T. D. Clemmer, G. J. Slotman, C. A. Metz, and R. A. Balk.** 1989. Sepsis syndrome: a valid clinical entity. Crit. Care Med. **17**:389–393.
3. **Calandra, T., J. D. Baumgartner, G. E. Grau, M. M. Wu, P. H. Lambert, J. Schellekens, J. Verhoef, M. P. Glauser, and The Swiss-Dutch J5 Immunoglobulin Study Group.** 1990. Prognostic values of tumor necrosis factor/cachectin, interleukin-1, interferon-α, and interferon-γ in the serum of patients with septic shock. J. Infect. Dis. **161**:982–987.
4. **Calandra, T., J. Gerain, D. Heumann, J. D. Baumgartner, M. Glauser, and The Swiss-Dutch J5 Immunoglobulin Study Group.** 1991. High circulating levels of interleukin-6 in patients with septic shock: evolution during sepsis, prognostic value, and interplay with other cytokines. Am. J. Med. **91**:23–28.
5. **Calandra, T., and M. Glauser.** 1990. Cytokines and septic shock. Diagn. Microbiol. Infect. Dis. **13**:377–381.
6. **Creasey, A. A., P. Stevens, J. Kenney, A. C. Allison, K. Warren, R. Catlett, L. Hinshaw, and F. B. Taylor, Jr.** 1991. Endotoxin and cytokine profile in plasma of baboons challenged with lethal and sublethal *Escherichia coli*. Circ. Shock **33**:84–91.
7. **Dofferhoff, A. S. M., V. J. J. Bom, J. van Ingen, H. G. de Vries-Hospers, B. P. C. Hazenberg, J. vd Meer, P. O. M. Mulder, and J. Weits.** 1991. Patterns of cytokines, plasma endotoxin, and acute phase proteins during the treatment of severe sepsis in humans, p. 43–54. *In* A. Sturk, S. J. H. van Deventer, J. W. ten Cate, H. Büller, L. G. Thijs, and J. Levin (ed.), Bacterial endotoxins: cytokine mediators and new therapies for sepsis. Wiley-Liss, Inc., New York.
8. **Dofferhoff, A. S. M., E. Vellenga, P. C. Limburg, A. van Zanten, and P. O. M. Mulder.** 1991. Tumour necrosis factor (cachectin) and other cytokines in septic shock: a review of the literature. Neth. J. Med. **39**:45–62.
9. **Exley, A. R., J. Cohen, W. Buurman, R. Owen, G. Hanson, J. Lumley, J. M. Aulakh, M. Bodmer, A. Riddell, S. Stephens, and M. Perry.** 1990. Monoclonal antibody to TNF in severe septic shock. Lancet **335**:1275–1277.
10. **Hack, C. E., E. R. De Groot, R. J. F. Felt-Bersma, J. H. Nuijens, R. J. M. Strack Van Schijndel, A. J. M. Eerenberg-Belmer, L. G. Thijs, and L. A. Aarden.** 1989. Increased plasma levels of interleukin-6 in sepsis. Blood **74(5)**:1704–1710.
11. **Halstensen, A., M. Ceska, P. Brandtzaeg, H. Redl, A. Naess, and A. Waage.** 1993. Interleukin-8 in serum and cerebrospinal fluid from patients with meningococcal disease. J. Infect. Dis. **167**:471–475.
12. **Hinshaw, L. B., P. Tekamp-Olsen, A. C. Chang, P. A. Lee, F. B. Taylor, C. K. Murray, G. T. Peer, T. E. Emerson, Jr., R. B. Passey, and G. C. Kuo.** 1990. Survival of primates in LD100 septic shock following therapy with antibody to tumor necrosis factor (TNF alpha). Circ. Shock **30**:279–292.
13. **Kreger, B. E., D. E. Craven, P. C. Carling, and W. R. McCabe.** 1980. Gram negative bacteremia. Reassessment of etiology, epidemiology and ecology in 612 patients. Am. J. Med. **63**:332–343.
14. **Michie, H. R., P. J. Guillou, and D. W. Wilmore.** 1989. Tumour necrosis factor and bacterial sepsis. Br. J. Surg. **76**:670–671.
15. **Morrison, D. C., and R. J. Ulevitch.** 1978. The effects of bacterial endotoxin on host mediation systems. Am. J. Pathol. **93**:527–617.
16. **Pinsky, M. R., J. L. Vincent, J. Deviere, M. Alegre, R. J. Kahn, and E. Dupont.** 1993. Serum cytokine levels in human septic shock: relation to multiple-system organ failure and mortality. Chest **103(2)**:565–575.
17. **Powell, A. C., L. A. Bland, C. W. Oettinger, S. K. McAllister, J. C. Oliver, M. J. Arduino, and M. S. Favero.** 1991. Lack of plasma interleukin-1β or tumor necrosis factor-α elevation during unfavorable hemodialysis conditions. J. Am. Soc. Nephrol. **2**:1007–1013.
18. **Rietschel, E. T., U. Schade, M. Jensen, H. W. Wollenweber, O. Lüderitz, and S. G. Greisman.** 1982. Bacterial endotoxins: chemical structure, biological activity and role in septicaemia. Scand. J. Infect. Dis. **31**(Suppl.):8–21.
19. **Tipple, M. A., L. A. Bland, J. J. Murphy, M. J. Arduino, A. L. Panlilio, J. J. Farmer III, M. A. Tourault, C. R. Macpherson, J. E. Menitove, A. J. Grindon, P. S. Johnson, R. G. Strauss, J. A. Bufill, P. S. Ritch, J. R. Archer, O. C. Tablan, and W. R. Jarvis.** 1990. Sepsis associated with transfusion of red cells contaminated with *Yersinia enterocolitica*. Transfusion (Arlington) **30**:207–213.
20. **Tracey, K. J.** 1991. Tumor necrosis factor (cachectin) in the biology of septic shock syndrome. Circ. Shock **35**:123–128.
21. **Waage, A., and A. O. Aasen.** 1992. Different role of cytokine mediators in septic shock related to meningococcal disease and surgery/polytrauma. Immunol. Rev. **127**:221–230.
22. **Waage, A., P. Brandtzaeg, A. Halstensen, P. Kierulf, and T. Espevik.** 1989. The complex pattern of cytokines in serum from patients with meningococcal septic shock. J. Exp. Med. **169**:333–338.
23. **Waage, A., A. Halstensen, and T. Espevik.** 1987. Association between tumour necrosis factor in serum and fatal outcome in patients with meningococcal disease. Lancet **i**:355–357.
24. **Ziegler, E. J., C. J. Fisher, Jr., C. L. Sprung, R. C. Straube, J. C. Sadoff, G. E. Foulke, C. H. Wortel, M. P. Fink, R. P. Dellinger, N. N. H. Teng, I. E. Allen, H. J. Berger, G. L. Knatterud, A. F. LoBuglio, C. R. Smith, and The HA-1A Sepsis Study Group.** 1991. Treatment of gram-negative bacteremia and septic shock with HA-1A human monoclonal antibody against endotoxin. N. Engl. J. Med. **324**:429–436.