Neutrophil Chemotaxis in Patients with Burns

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In a group of 22 patients with second and third degree burns, seven were found to have impaired chemotaxis. The chemotactic defect was present from two to 68 days and eventually became normal. The impairment was found to be due to a primary transient defect in polymorphonuclear leukocytes and not to an inhibitor or inactivator in the serum.

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BACTERIAL INFECTIONS AND SEPSIS still are the most dreaded complications of the traumatic insult sustained by severe thermal injuries and, in spite of the topical and systemic applications of a variety of antibiotic regimens, they remain the leading cause of death. A number of conditions, such as disruption of the integrity of the skin, increased caloric requirement in the presence of decreased intake, loss of plasma, fluid and electrolytes, all contribute in various degrees to the establishment and dissemination of infection. The role of the host's humoral[1] and cellular immunological defense mechanisms has recently been appreciated. Defective polymorphonuclear (PMN) killing[2] and chemotaxis[3–5] have been described and associated with the clinical status and ultimate outcome of the patients. The present study was undertaken to evaluate further PMN chemotactic function as a contributing factor in the host's defense against severe thermal injuries.

Material and Methods

Patients admitted to the Burn Unit with second and/or third degree burns were included in the study. Blood was drawn into heparinized syringes and white blood cells were separated with a mixture of Methocel-Isopaque[6] and washed with tissue culture medium 199 (TC-199). A final suspension of 2.5×10^6 PMN/ml in two per cent Bovine Albumin was made. Chemotactic factor was generated from sera of patients with burns as well as normal subjects by incubating 0.1 ml of sera with one milligram of Zymosan and then bringing up the total volume to one milliliter with TC-199.[7] Chemotaxis was measured by a previously described modification of Boyden assay.[7,8] In brief, 2.5×10^6 PMN in two per cent Bovine Albumin was placed in the upper compartment of the modified Boyden chambers and separated from the chemotactic agent by means of micropore filters (pore size of 5 μ). After three hours of incubation at 37° the chambers were disassembled, the filters separated, fixed and stained by routine histologic methods.[7] They were then examined under the microscope and final evaluation was made by taking the average counts in five random fields of the number of neutrophils found on the lower surface of the filter. Similar measurements were made in filters obtained from control chambers without chemotactic factors. Everytime the chemotaxis was tested, PMN cells from a normal control was included. The day to day variability between tests sequentially in a single control ranged between five to 15%. Qualitative nitroblue tetrazolium (NBT) reduction was determined according to the previously described technic.[9]

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Submitted for publication: January 12, 1977.

Results

A total of 22 patients between the ages of 12–82 years were examined. There were 16 males and six females. Twelve of the patients had second degree burns ranging from five to 35% of total body surface. Four patients were classified as only third degree burns (five to 15%) and the other six had combination of second and third degree burns (five to 30% second degree and 12.5 to 50% third degree). Twenty-one of the patients in this group recovered completely with or without eventual skin grafting. Only one patient, a 24-year-old male with 30% second degree and 50% third degree burns and inhalation injury died.

A variety of bacteria—*Pseudomonas aeruginosa, Staphylococcus aureus*, multiple organisms, and fungi (candida) were grown from the burn lesions. Blood cultures were universally negative and none of the patients showed clinical signs of generalized sepsis.

TABLE 1. *PMN Chemotaxis, Number of PMN/HPF – Mean ± S.E. (Range)*

	TC-199	p‖	Zy/Cs†	p	Zy/Bs‡	p	NBT§	p
Burn Patients (15)*	3.8 ± 0.4 (1–8)		94.7 ± 10.9 (42–223)		92.2 ± 11.2 (34–209)		32.8 ± 17.5 (4–71)	
		>0.5		>0.5		>0.5		<0.001
Normal Controls (32)	3.5 ± 0.2 (1–6)		104.7 ± 10.0 (30–289)		109.3 ± 12.4 (48–187)		6.2 ± 3.2 (2–10)	

* Number of subjects tested in parenthesis.
† Zymosan in pooled control sera.
‡ Zymosan in serum from burn patients.
§ Percent of NBT positive PMN.
‖ Paired sample student's t test.

Patients received a variety of sedatives, topical and/or systemic antibiotics, antipyretics and analgesics.

Fifteen of the patients had normal PMN chemotaxis. Ten of these had only second degree burns (two to 35%), three had third degree burns (five to 15%) and two had a combination of second and third degree burns. The PMN chemotaxis in all of these patients was initially within the normal range and remained so up to the time of their discharge. The chemotactic response of the PMN cells from these patients to the stimulus generated by the addition of Zymosan to a pool of normal sera was 94.7 ± 10.9 PMN/HPF (range 42–223) and compared favorably ($p > 0.5$) with the response of PMN's from normal controls 104.7 ± 10 PMN/HPF (range 30–289). Similarly normal PMN's as well as PMN's from the above patients were equally responsive to the stimulus generated by the addition of Zymosan to sera from subjects with burns. These results are summarized in Table 1.

In contrast seven patients had abnormal PMN chemotaxis. Two of these patients had second degree burns (22% and 35% respectively), one had only third degree burns (five per cent), and the other four had mixed second and third degree burns. The abnormal PMN chemotaxis lasted from at least two to 68 days and eventually returned to values within the normal range. Only one patient who had severe skin burns and inhalation injury died two days after admission while his PMN chemotaxis was abnormal. These results are summarized in Table 2.

The simple qualitative NBT test was initially found to have a higher (15–71%) than normal value (two to 10%) in all of the burn patients with chemotactic defect. The high NBT values correlated with the superficial infection of the skin in the burned areas and had no correlation with the PMN chemotactic findings.

Discussion

The immediate prevention and treatment of shock in patients with severe thermal injuries has reduced the initial mortality. However, mortality associated with severe infections due to the presence of gram negative bacteria in the burn eschar and due to increased susceptibility to infections remains a major problem. It is at that point that the host's defense mechanisms may play a major role. The neutrophils from patients with severe burns have been found to be deficient in their ability to kill ingested bacteria[10] as well as in their chemotactic responses.[3,5] While a unified explanation for these findings is not available, Edelson's[11] suggestion that the chemotactic abnormalities are membrane associated while killing defects involve specific metabolic pathways seem quite reasonable.

The pathophysiology of decreased PMN chemotaxis remains to be elucidated. Sera from patients with various degree of burns did not contain chemotactic inactivators since their ability to generate Zymosan

TABLE 2. *Summary of Burn Patients with Abnormal PMN Chemotaxis*

Patient	%—Degree Burn	Chemotaxis PMN/HPF TC-199	Zy/Cs	Zy/Bs	Days After Burn	NBT
E.B.	22%—Second	3	3	5	2	37
		3	47	58	7	20
L.S.	5%—Third	2	12	2	13	15
		1	2	3	28	N.D.
		3	45	49	35	33
C.Mc.	26%—Second	4	19	7	16	N.D.
	24%—Third	3	2	7	28	30
		4	4	4	36	19
		1	3	3	50	21
		3	21	28	68	12
		5	73	58	80	10
W.P.	15%—Second	5	7	9	2	N.D.
	30%—Third	2	2	2	10	N.D.
		2	2	3	17	56
		4	2	5	24	48
		2	12	5	31	60
		4	69	92	38	22
P.C.	35%—Second	2	5	3	2	60
		1	2	6	4	40
		3	15	53	10	22
W.D.	40%—Second	1	5	5	4	71
	20%—Third	1	3	3	15	30
		0	3	3	24	N.D.
		5	53	29	38	24
J.B.	Skin & Inhalation	2	6	0	2 (died)	N.D.
Controls		(1–6)	(30–289)	(48–187)		(2–10)

induced chemotactic activity was not different from that of normal pooled sera. Similarly the addition of "burn serum" to the upper compartment of the Boyden's chamber did not inhibit the subsequent chemotactic migration of the PMN. Therefore, the abnormal chemotaxis is primarily a PMN defect.

Yoshida[12] and his associates isolated a chemotactic factor from burned skin lesions and called it "leucoegressin". The factor can be produced *in vitro* and *in vivo* by the addition of neutral sulfhydryl dependent proteases from inflammatory tissue to the serum IgG. It is possible that after responding to an *in vivo* chemotactic agent the PMN cells become "deactivated" and fail to respond to further chemotactic stimuli *in vitro*. It is also possible that *in vitro* impairment of chemotaxis may follow *in vivo* phagocytosis of a variety of tissue particles following the thermal injury. The phagocytic process utilizes the limited energy resources of the cells[13] and may render them chemotactically inactive. The increased NBT values found in our patients may indicate increased phagocytic activity. However, not all patients with increased NBT values had decreased chemotaxis.

Finally, Allgöwer[14] and his colleagues isolated a specific toxic factor, "burn toxin" from the skin of burn patients. This factor was found to be harmful to cell wall membranes. It is possible that the "burn toxin" in limited amounts may induce membrane changes that may culminate in impaired chemotaxis and in more severe cases may cause phagocytic and killing defects. Whatever the pathophysiology may be, the development of PMN chemotactic defect may eventually lead to defects in host's resistance against infection and may contribute to causes of generalized sepsis.

Acknowledgment

The authors gratefully acknowledge the help of Dr. I. C. Song and Dr. S. Kountz who allowed study of his patients, and Ms. Judy Arcoleo for typing the manuscript.

References

1. Alexander, J. W. and Moncrief, J. A.: Immunologic Phenomena in Burn Injuries. JAMA, 199:105, 1967.
2. Alexander, J. W., Hegg, M. and Altemeier, W. A.: Neutrophil Function in Selected Surgical Disorders. Ann. Surg., 168:447, 1968.
3. Alexander, J. W. and Wixson, D.: Neutrophil Dysfunction and Sepsis in Burn Injury. Surg. Gynecol. Obstet., 130:431, 1970.
4. Allgöwer, M., Städtler, K. and Schoenberger, G. A.: Burn Sepsis and Burn Toxin. Ann. R. Coll. Surg. Engl., 55:226, 1974.
5. Boyden, S.: The Chemotactic Effect of Mixtures of Antibody and Antigen on Polymorphonuclear Leucocytes. J. Exp. Med., 115:453, 1962.
6. Bøyum, A.: Separation of White Blood Cells. Nature, 204: 793, 1964.
7. Edelson, P. J., Stites, D. P., Gold, S. and Fudenberg, H. H.: Disorders of Neutrophil Function. Defects in the Early Stages of the Phagocytic Process. Clin. Exp. Immunol., 13:21, 1973.
8. Fikrig, S. M., Karl, S. C., Rauscher, G. et al.: Chemotaxis in Burns (Abstract). Pediatr. Res., 9:329, 1975.
9. Greene, W. H. and Quie, P. G.: The Role of Neutrophils in Fighting Off Bacterial Infections. Med. Opinion, 4:48, 1975.
10. Park, B. H., Fikrig, S. M. and Smithwick, E. M.: Infection and Nitroblue-Tetrazolium Reduction by Neutrophils. Lancet, ii:532, 1968.
11. Sbarra, A. J. and Karnovsky, M. L.: The Biochemical Basis of Phagocytosis. I. Metabolic Changes During the Ingestion of Particles by Polymorphonuclear Leukocytes. J. Biol. Chem., 234:1355, 1959.
12. Ward, P. A., Cochrance, C. G. and Müller-Eberhard, H. J.: The Role of Serum Complement in Chemotaxis of Leukocytes In Vitro. J. Exp. Med., 122:327, 1965.
13. Warden, G. D., Mason, A. D. Jr. and Pruitt, B. A. Jr.: Evalution of Leukocyte Chemotaxis In Vitro in Thermally Injured Patients. J. Clin. Invest., 54:1001, 1974.
14. Yoshida, K., Yoshinaga, M. and Hayashi, H.: Leucoegressin. A Factor Associated with Leucocyte Emigration in Arthus Lesions. Nature, (Lond.), 218:977, 1968.