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Clinical significance of leukocyte infiltrative response in deep wound of patients with major burns

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Abstract

Objective: To investigate the time course of infiltrated leukocytes and the subpopulations of infiltrated lymphocytes in deep wounds, and their relationship with injury severity in seriously burned patients.
Methods: Six patients with major burns were enrolled in the study. Specimens were taken from deep partial-thickness burn wounds of all patients at 3 days, 1 week, 2 weeks and 3 weeks after burn. The appearance time and cellular components of infiltrated leukocyte zone in the burn wound were evaluated by histological and immunohistochemistry examination with lymphocyte monoclonal antibodies.
Results: The infiltrated leukocyte zone of burn wound formed 1–2 weeks after burn. The more severe the degree of injury (including burn area, depth and combined injuries) was, the later the infiltrated leukocyte zone appeared. The infiltrated cells mainly consisted of polymorphonuclear leukocytes (PMN) during the early period after burn while macrophages and lymphocytes appeared later. There were some changes of the T lymphocyte subsets and their activation degree in the burn wound.
Conclusion: The changes of infiltrated leukocyte zone of wound following burns were closely related to the injury severity, and represented alteration of the anti-infection ability and immune rejection of local wound. These results provide important evidence for appropriate wound treatment and prolonging the survival of skin allograft.

Keywords: Leukocyte infiltrative response; Burn wound; Major burns

1. Introduction

The burn wound causes not only local changes but also systemic pathophysiological disorders in major burn patients. The management of extensive burn wounds, especially deep burn wounds, is one of the critical steps in the successful burn treatment [1]. Infection is a leading cause of death in severe burn patients, and the burn wound is the most common site of infection after burns [2–4]. Immune dysfunction after burn predisposes patients to sepsis and multiple organ failure leading to increased mortality [5–7]. The burn wound is divided into three zones, i.e. coagulation, stasis and congestion, and hyperemia [8]. Fluid from burn wounds, such as blister fluid and subeschar tissue fluid, has suppressive effects on normal neutrophils and lymphocytes [9,10]. These findings support the concept that cellular function can be altered by the microenvironment of burn wound in which the cells are bathed. However, few data are available about the local cellular response of the burn wound, even though burn wound sepsis is a common cause of death in the burn victims. Therefore, it is necessary to characterize the infiltrated leukocytes response in the burn wound of patients suffering from severe burn injuries. This will bring more help in the interest of clinical wound care.

The wounds of first-degree burn or superficial partial-thickness burn often complete healing within 3–5 days or 5–14 days, respectively. A full thickness wound will not heal without grafting [11]. The deep partial-thickness burns affect the deep dermis, healing is usually completed

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within 3 weeks, though it may take up to 6 weeks. The acute inflammatory process and subsequent infection can increase the depth of tissue destruction in the burn wound, converting deep partial-thickness wounds to full-thickness wounds that ultimately require skin grafting. Obviously, the local cellular response of deep partial-thickness wounds might determine different outcomes between spontaneous healing and the need for skin grafting [12]. Meanwhile, severe inflammatory response syndrome may occur with burns involving more than 30% total body surface area (TBSA), and become more severe with larger burns. This might also affect the local cellular response of burn wound. The full-thickness burn wound is usually excised at an early stage after burn. For deep partial-thickness burns, the wound will not be grafted until skin autografts are available and the wound is still open at 3 weeks after burn. Based on these considerations, we addressed this 3-week-endpoint study on the leukocyte infiltrative response in deep partial-thickness wound of patients with major burns. Therefore, a pilot and prospective study was conducted in six seriously burned patients with burned TBSA over 30%. The dynamic changes of infiltrated leukocytes and lymphocyte subpopulations in deep partial-thickness wound were observed, and their correlation with the burn severity was also analyzed.

2. Clinical data and methods

2.1. Clinical data

Inclusion criteria included age between 18 and 65 years, admission to hospital within 24 h postburn and burn size of more than 30% TBSA with at least 1% deep partial-thickness burn area, assessed by clinical manifestation of burn wound. Six patients who met the criteria at Clinical Division, Institute of Burn Research, Southwest Hospital were enrolled in the study. The age range was from 18 to 38 years (mean, 28.5 ± 9.0 years). The burnt TBSA was $62.3 \pm 24.8\%$, and the burnt full-thickness burn TBSA was $19.5 \pm 32.5\%$. Patients were assigned to either moderate group or severe group according to severity of injury which were evaluated by total burn area, deep burn area and combined injuries [13]. There were three patients with burned TBSA of 35–60% and deep burn of 13–25% in the moderate burn group, and another three patients with burned TBSA of 55–94% and deep burn of 50–89% in severe burn group (Table 1). The burn wounds were treated with topical silver sulfadiazine and exposure therapy. After burn resuscitation, the patients underwent staged early burn wound excision and intermingled or microskin grafting with autografts, allografts, or both for deep burn wounds. Punch-biopsy specimens were taken from the deep partial-thickness burn wounds of all patients at 3 days, 1 week, 2 weeks and 3 weeks postburn to assess leukocyte infiltration of burn wound. The Ethics Committee of Southwest Hospital approved the investigative protocol.

2.2. Observed indexes and their methods

All wound tissue samples were divided into two parts for histopathologic and immunohistochemical examinations, respectively. One was fixed in 10% neutral formalin, embedded in paraffin, cut into 4 μm sections and stained with hematoxylin and eosin. Another part was stored in liquid nitrogen. The frozen tissues were sectioned at 6 μm and stained with ABC (avidin and biotinylated peroxidase complex, ABC) kit (Vector) and lymphocyte monoclonal antibodies (Wuhan Institute of Biological Products) which included CD3, CD4, CD8, CD25 (IL-2 receptor α, IL-2R α) and CD19. Briefly, the frozen sections were fixed in cold acetone, incubated with 1% H_2O_2 and normal rabbit serum at room temperature to block endogenous peroxidase and nonspecific binding sites, respectively. After washing in PBS, the sections were incubated with 1:10–1:20 diluted primary monoclonal antibody at 4 °C overnight. Following a brief wash, a secondary biotinylated antibody was added. After appropriate incubation and washing, the avidin–biotin peroxidase complex was applied. A chromogen solution of 3,3′-diaminobenzidine (DAB) was used. Following chromogen development, the sections were counterstained briefly in Gill's hematoxylin, dehydrated through graded alcohols to xylene, coverslipped, and examined microscopically. Sites of primary antibody attachment were identified by the presence of an insoluble brown reaction product.

Table 1
Clinical data of severely burned patients

Patient number	Sex	Age (years)	%TBSA burned (superficial II°/deep II°/III°)	Combined injury	Prognosis	Severity
1	M	35	35 (22/12/1)	No	Cure	Moderate
2	M	19	40 (15/20/5)	No	Cure	Moderate
3	M	36	60 (35/24/1)	No	Cure	Moderate
4	M	25	94 (5/4/85)	Mild inhalation injury	Death	Severe
5	F	18	55 (5/40/10)	Craniocerebral injury, fracture	Cure	Severe
6	M	38	90 (20/55/15)	No	Cure	Severe