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Alternations of surface antigens on leukocytes after severe injury: correlation with infectious complications

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Abstract *Objective:* To investigate the alternations of surface antigens of leukocytes after severe injury and the correlation with clinical outcome.
Setting: Emergency Department and Intensive Care Unit of a university hospital.
Patients: Patients with severe trauma (injury severity score > 16) were enrolled. Those who were transferred or had critical injuries were excluded.
Measurements and results: Polymorphonuclear cells (PMN) and mononuclear cells (MN) were isolated from patients on the 1st, 3rd and 7th day following injury. The mean fluorescent expressions of CD11b and CD16 of PMN, and CD25 of MN were measured and compared with those obtained from paralleled controls. Sixteen injured patients were included. The CD11b expressions of PMN increased on the 1st day and were still high on the 7th day. The CD16 expressions decreased on the 1st day and CD25 decreased on the 3rd day; both were still low on the 7th day. Six patients developed infectious complications. CD11b expression remained high and CD16 expression remained low on three measurements of the infectious patients, whereas both expressions recovered on the last measurement of non-infectious patients. CD25 expression remained low in both groups. Three infectious patients with pneumonia died from mutiple organ failure.
Conclusion: Phenotypic alternations of leukocytes develop early after severe injury. The alternations may represent a state of activation of PMN and subsequent suppression of IL-2 related immunity. Persistent activation of PMN with enhanced CD11b and attenuated CD16 expression indicates the development of infectious complications and a poor prognosis can be anticipated if the infectious sites can not be controlled early.

Key words Polymorphonuclear cells · Mononuclear cells · CD11b · CD16 · CD25 · Trauma

Introduction

Many studies have shown alternations of leukocyte functions with systemic non-discriminant hyperinflammation after injury [1–3]. The defense mechanisms of host leukocytes involve various cell surface receptors that mediate cellular activation, adhesion, phagocytosis and intercellular reactions. Among them, receptors for IgG (FcrR) or complement (CR) are closely related to neutrophil functions [4, 5]. On their surface human polymorphonuclear leukocytes (PMN) have an adhesive glycoprotein, which is identified as a receptor for C3bi (CR3). CR3, one of the β2-integrins (Mac-1), is a heterodimer composed of an α-chain (CD11b) and a β-chain (CD18). CD11b/CD18 is of primary importance in mediating neutrophil adhesion, aggregation and phagocytosis. Various receptors for IgG are also present on the PMN surface. One of these receptors, FcrR III (a

low affinity receptor designed CD16), is closely related to the phagocytosis and other unknown functions of PMN. Severe burn injury may cause alternating expressions of receptors with functional changes of PMN [6].

As well as alternations of PMN functions, dramatic derangements of cell-mediated immunity have frequently been reported after trauma. The most striking abnormality is the decrease in interleukin-2(IL-2) production [7]. IL-2, also known as T cell growth factor, is a glycoprotein produced by activated T lymphocytes (Th 1 cells). IL-2 regulates the development of immune response to antigen in an autocrine or paracrine fashion by interaction with its receptors (IL-2R) expressed on the surface of mononuclear cells (MN).

Our study tried to evaluate the alternations of leukocyte receptors, including FcrR III (CD 16) and CR3 (α-chain of Mac-1, CD11b) on PMN and IL-2R (p55, CD25) on MN, from patients with severe injury and to assess their correlation with clinical outcome.

Patients and methods

Patients

Patients with blunt multiple traumas and injury severity scores (ISS) above 16 who needed intensive care were consecutively included in our study [8]. Not included were patients transferred from other hospitals or those with such critical injury that long-term survival was not expected. The initial resuscitation and management of patients were performed according to the guidelines of ATLS provided by the American College of Surgeons. Three venous blood samples were obtained on the 1st (24 h), 3rd (72 h) and 7th day (168 h) following injury. Healthy and age-matched volunteers were used as controls. The presence or absence of infectious complication was determined by using the Centers for Disease Control (CDC) guidelines [9]. The study was conducted according to the principles established in Helsinki.

Cell preparations

Peripheral venous blood was collected in EDTA vacutainers. Red blood cells were removed by Dextran (6.0% wt/vol, 242000; Sigma) sedimentation. Cell-rich plasma was layered over Ficoll-Paque (Pharmacia, LKB) and centrifuged at 400xg for 30 min [10]. MN was collected from the interface and granulocytes from the bottom of the solution. The cells were then washed in phosphate buffered saline (PBS, pH 7.4, Sigma) and suspended in RPMI 1640 cell culture medium with 2.5% (wt/vol) bovine serum albumin (Sigma). Cell viability was tested by trypan blue exclusion and always above 95%. The final cell concentration was adjusted to 2.0×16^6/ml for the studies of PMN and 0.5×10^6/ml for MN.

Monoclonal antibody staining

Monoclonal antibody recognized CD 16 (anti-human 11a, FITC conjugated) and CD11b (Leu 15, phycoerythrin conjugated) were used for the labelling of PMN. Monoclonal antibody recognized CD 25 (anti-IL-2R, FITC conjugated) was used for the labelling of MN. In brief, aliquots (100 μl) of cell suspensions were mixed with 50 μl of fluorescent monoclonal antibodies. The mixtures were incubated for 20 min at room temperature and washed with PBS, then resuspended in 1 ml of paraformaldehyde solution (0.5% wt/vol PFA and 2.0 mmol/l EDTA; Sigma) for running under flow cytometry. Before the staining of CD 25, MN were incubated with pure phytohemagglutinin (PHA, 1 mg/ml, Sigma) for 24 h. The fluorescent monoclonal antibodies used in our study were the products of the Becton Dickinson (San Jose, CA).

Flow cytometry analysis

Analysis was performed in a Becton Dickinson FACS can supported by a Hewlett-Packward 9000 computer (Model 310) with a computer soft ware package (Consort 30). Gating on populations of PMN or MN were performed according to criteria of forward and right angle scatter. Data were collected from 10,000 events for PMN, and 5,000 for MN, study. The mean channel fluorescence was recorded and analyzed. The final results were normalized with paralleled controls.

Statistical analysis

Mean fluorescent expressions of leukocyte antigens from the patients were described as percentages of those from the paralleled controls. Wilcoxon paired sign – rank test was used for comparison with controls. For two independent groups, the Mann-Whitney test was performed. For three groups, Kruskal-Wallis following by Mann-Whitney test were performed. For the nominal data, Fisher exact test was used. For investigating correlation between two single variables, Spearman correlation was performed. A p of less than 0.05 was considered to be statistically significant.

Results

From August 1993 to September 1994, 16 patients with blunt multiple trauma were studied. There were nine male, and seven female, patients ranging in age from 18 to 67 years (41.4 ± 18.5; mean ± SD), ISS from 18 to 41 (24.7 ± 5.9) and hospital days from 15 to 48 (25.0 ± 10.1). Sites of injury were seven heads, six chests, seven abdomens, and eight bones. Nine patients underwent ten operations during the study. Six laparotomies were performed on the 1st post-injury day, and four orthopedic procedures were carried out: one on the 1st, two on the 3rd and one on the 5th post-injury day. Two patients had early multiple organ dysfunction that developed on post-injury day 3; both needed ventilator support and had mild impairment of renal function. Six patients developed infectious complications during hospitalization (infectious group). Three patients had pneumonia; clinical presentation of fever and pulmonary infiltration developed from the 9th post-injury day in two and the 6th in one patient. Two patients had intra-abdominal abscesses; their symptoms presented from the 8th and 9th post-injury day, respectively. One patient developed wound infection on post-injury day 7. The three patients

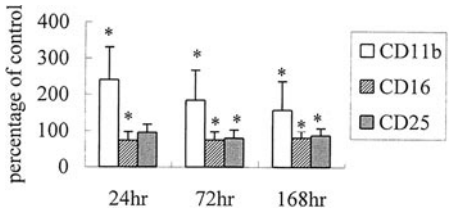

Fig. 1 CD11b, CD16 of PMN and CD25 of MN in 16 patients. Mean fluorescent expressions were expressed as percentage of those of controls. * $p < 0.05$ compared with controls

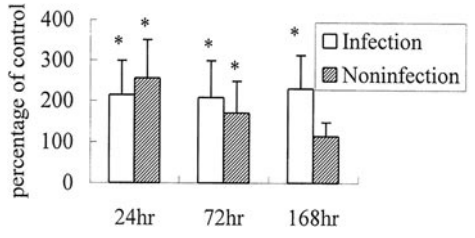

Fig. 2 Mean CD11b expression of PMN in infectious ($n = 6$) and non-infectious ($n = 10$) patients. * $p < 0.05$ compared with controls

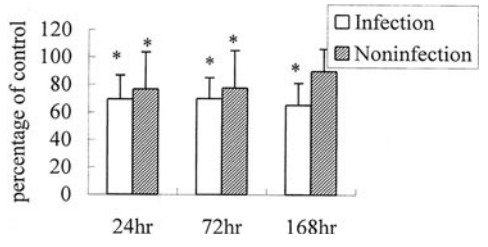

Fig. 3 Mean CD16 expression of PMN in infectious ($n = 6$) and non-infectious ($n = 10$) patients. * $p < 0.05$ compared with controls

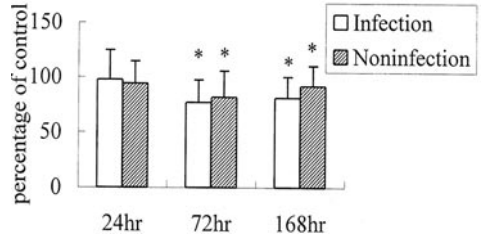

Fig. 4 Mean CD25 expression of MN in infectious ($n = 6$) and non-infectious ($n = 10$) patients. * $p < 0.05$ compared with controls

with pneumonia suffered from multiple organ failure thereafter and expired on the 21th, 18th and 19th post-injury day, respectively. The other infectious patients survived after drainage of the infectious sites. All non-infectious patients recovered uneventfully (non-infectious group). There was no significant difference between the infectious and non-infectious groups with regard to age (50.5 ± 19.6 vs 36.0 ± 16.5), ISS (25.3 ± 8.4 vs 24.3 ± 4.2) and patients with initial shock in the emergency room (33.3 % versus 30 %).

Mean fluorescent expressions for cell surface antigens, described as the percentage of those obtained from controls with parallel measurements, are shown on Fig. 1. The expression of the CD11b on PMN increased immediately at 24 h and remained high at 168 h. The expression of CD16 decreased immediately at 24 h and remained low at 168 h. The CD25 of MN decreased after 72 h and remained low at 168 h. Further analysis was performed between those of the infectious and non-infectious groups. In the study of PMN, CD11b expression remained high in the infectious group but in the non-infectious group it was not significantly different from that of the controls after 168 h (Fig. 2). On the other hand, CD16 expression remained low in the infectious group and there was no significant difference from the controls in the non-infectious group after 168 h (Fig. 3). In the study of MN, CD25 expression remained low in both groups compared to the controls. Although CD25 expression was higher in the non-infectious group than in the infectious group at 168 h, there was no significant difference (Fig. 4). The correlation between ISS and the mean fluorescent expressions of various surface antigens in all three measurements was not significant.

Discussion

Two subclasses of human FcrR III, hFcrR IIIA and hFcrR IIIB, have been identified [5]. Neutrophils appear to express only the PI-link subclass of FcrR III [11]. Upon activation of neutrophils, these receptors are released into the plasma by a proteolytic mechanism [12, 13]. Soluble FcRIII may have a modulatory function on the production of IgG by B lymphocytes [14, 15]. The depressed CD16 expression of neutrophils after severe injury in our study represented a state of cellular activation and systemic inflammatory response immediately following trauma. The mean CD 16 expression of PMN decreased following severe injury and remained low in the infectious patients, whereas it recovered in non-infectious patients after 168 h. Although increased serum FcrIII may have some immunomodulatory effects, suppressed CD16 expression of PMN will result in decreased IgG-opsonized phagocytosis, which may be detrimental for patients with sepsis [16].

As a complement receptor for C3bi, CR3 is also recognized as a member of the integrin family that mediates intercellular reactions. The importance of integrin (CD11/CD18) on cellular function has been demonstrated by recurrent bacteria infection found in patients with inherited deficiency of Leu-CAM (CD11/CD18) [17]. Ligands for the CD11/CD18 also present on the endothelium with ICAM-1 (CD54, cytokine in-

duced) [18]. The upregulation of CR3 expression on the cell surface may represent a state of PMN degranulation [19]. Several studies have shown that enhanced CR3 on PMN induced cellular aggregation, adhesion and release of products that are detrimental to the endothelium. The impaired endothelium may be responsible for the development of ARDS after trauma [20, 21]. From our study, the CR3 expression of PMN enhanced immediately after injury. Although CR3 expression has been reported to correlate with burn surface area, we did not found a significant correlation with the injury severity score after blunt trauma [16]. The CD11b expression on PMN returned to normal 1 week after injury in non-infectious patients, whereas it remained high in infectious patients. Persistent enhancement of CD11b may imply subsequent stimulation from infectious sites following the initial traumatic insult. The subsequent stimulation, which may be mediated by cytokines, re-activates circulating PMN. The activated PMN is important in the defense process of bacterial invasion. However, the subsequently activated PMN may induce an intense systemic inflammatory response resulting in so-called second hit injury that culminates in multiple organ failure through PMN-mediated injury [22].

Major burn or surgery results in decreased production IL-2 [9, 23]. A recent study showed that transmembrane signalling caused impairment of IL-2 production and emphasized that the mediators released after injury played important roles [24]. IL-2 regulates the development and control of an immune response to antigen in an autocrine or paracrine fashion through the interaction with its high affinity receptor (IL-2R) expressed on the surface of diverse target cells. After the activation of lymphocytes, the IL-2 transcription appears before that of IL-2R [25]. The presence of receptors on cells is necessary for the action of IL-2. The decreased CD25 expression of MN indicates impaired IL-2 related cellular immunity. Our study showed significant suppression of CD25 on MN from the 3rd day following severe injury. Although the CD25 measurement of both groups was less than that of the controls, the non-infectious group seemed to have more than the infectious group in the last measurement.

Our study showed that severe injury creates an environment of immunological derangement characterized by the initial activation of PMN and subsequent suppression of IL-2 related immunity. Possibly many cytokines were involved in their interactions. Both over-activation of PMN and suppression of IL-2 immunity would make the host vulnerable to end organ damage and infection. If patients did not become infected, the abnormality recovered. On the other hand, when the patients developed infection, the unfavorable environment persisted and culminated in multiple organ failure if the infectious sites could not be controlled rapidly. Further study may be needed to clarify whether the immunological derangement induces infectious complications or infectious complications cause an abnormal immune response with alternating phenotypes of leukocytes. However, our patients had persistent phenotypic alternations that coincided with or preceded the clinical manifestations of infectious complications. Persistent alternations of leukocytic phenotypes provided early signs of infection after injury. Poor prognosis was also found in patients whose infectious foci could not be controlled early on a state of persistent PMN activation and depressed IL-2 related immunity.

In conclusion, phenotypic alternations of leukocytes develop early after severe injury. The alternations represent a state of activation of natural immunity (PMN) and subsequent suppression of IL-2 dependent immunity. Those changes of PMN correlate with infectious complications. Persistent activation of PMN with enhanced CD11b and attenuated CD16 expression indicates the development of infectious complications and a poor prognosis may be anticipated if the infectious sites can not be controlled early.

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