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Regulation of Fas-Mediated Apoptosis in Neutrophils after Surgery-Induced Acute Inflammation

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Background. **Neutrophils undergo rapid Fas-mediated apoptosis during *in vitro* culture. The purpose of this study was to investigate the effects of surgical stress upon the Fas-mediated apoptotic response in circulating neutrophils.**

Materials and methods. **Blood samples were drawn from eight patients with a mandibular prognathism, and who had undergone a bilateral sagittal split ramus osteotomy, at 2 days before, and at 1 and 5 days after surgery. The circulating neutrophils in each blood sample were then evaluated for their susceptibility to Fas-mediated apoptosis in either the presence or the absence of autogenous plasma.**

Results. **Fas-induced apoptosis in the neutrophils of these surgically treated patients was found to be slightly accelerated at 1 day postoperatively in the presence of FBS, compared with 2 days preoperatively and 5 days postoperatively. However, we obtained different results for these experiments in the presence of autogenous plasma. The Fas-induced apoptotic response levels in the neutrophils at day 1 postsurgery following exposure to autogenous plasma were significantly suppressed compared with the levels at both 2 days preoperatively and 5 days postoperatively. The Fas expression levels on the cell surface of the neutrophils were not altered, but the levels of soluble Fas (sFas) in the plasma were reduced to almost inverse levels during the postoperative periods. The levels of granulocyte-macrophage colony-stimulating factor, interleukin-6, and interleukin-8 levels in the plasma were also markedly raised in the plasma from each of these patients at 1 day postoperatively. However, the anti-apoptotic effects of the plasma on the Fas-mediated neutrophil apoptosis were not influenced by the addition of their neutralizing antibodies for these cytokines. The suppressive effects of postoperative plasma on Fas-mediated neutrophil apoptosis were blocked by the phosphatidylinositol 3-kinase (PI 3-K) inhibitors, LY294002, and wortmannin. Additionally, these effects were also abrogated by the extracellular signal-regulated kinase (ERK) inhibitor, PD98059, but not by the p38 mitogen-activated protein kinase inhibitor, SB203580.**

Conclusions. **The increase in sFas levels in the plasma of patients with acute inflammation may lead to the inhibition of Fas-mediated neutrophil apoptosis. Moreover, the activation of the PI 3-K and ERK signaling-dependent pathways may, in part, also contribute to the down-regulation of the Fas-mediated apoptotic response in neutrophils.**

Key Words: **neutrophils; Fas; apoptosis; surgical stress; inflammation; phosphatidylinositol 3-kinase; extracellular signal-regulated kinase; soluble Fas.**

INTRODUCTION

The cellular events of acute inflammation are heralded by the tissue influx of large numbers of neutrophils. These cells have a well-established potential to injure tissues by a variety of mechanisms, and they have been implicated in the pathogenesis of numerous inflammatory diseases in several organs [1, 2]. However, it is also clear that acute inflammation has evolved as part of a beneficial host response to injury and infection that normally resolves with minimal residual tissue damage [3, 4]. By contrast, as for the initiation phase of acute inflammation, the resolution is poorly understood. Neutrophils must act without

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discharging their toxic contents at inflammatory sites for the normal resolution of acute inflammation. In fact, neutrophils disappear from inflamed sites without expanding tissue injury in the resolution stage of acute inflammation.

Previous studies have shown that apoptosis of neutrophils represents a physiological clearance mechanism in the circulation and in the tissue to create and maintain the homeostasis of neutrophil numbers [5, 6]. Apoptosis, which represents an alternative fate to necrosis, not only determines neutrophils uptake by macrophages, but also is associated with a loss of neutrophil function, such as chemotaxis, phagocytosis, degranulation, and respiratory burst [7, 8]. On the contrary, because the reduction of neutrophil apoptosis is concomitant with retained neutrophil functions such as respiratory burst activity and chemotaxis [9], the down-regulation of physiological neutrophil apoptosis may considerably augment the potential for tissue injury due to the release of toxic metabolites.

The Fas-Fas ligand (FasL) system is recognized as a major pathway for the induction of apoptosis in cells and tissues, and for the removal of unwanted cells in the human body [10, 11]. The finding that human neutrophils express functional Fas suggests that this molecule has emerged as a critical pathway for the induction of neutrophil apoptosis, and may be involved in the regulation of acute inflammatory diseases [12, 13]. It is well known that spontaneous and Fas-mediated apoptosis in neutrophils can be regulated by various inflammatory related mediators, including pro-inflammatory cytokines *in vitro* [9, 14, 15]. Surgical stress has also been shown to induce a remarkable inflammatory response and to up-regulate the pro-inflammatory cytokine production and number of neutrophils [16, 17]. Furthermore, previous studies have shown that the plasma or serum of patients with inflammatory diseases suppresses neutrophil apoptosis [18-24]. These results suggest that the predominance of anti-apoptotic signals leads to a serious pathophysiological state. However, the mechanisms of regulation for neutrophil apoptosis on the Fas-FasL system remain unknown during the perioperative periods.

Mitogen-activated protein kinases (MAPKs) play a significant role in controlling the cell death machinery. In general, activation of p38MAPK promotes cell death and extracellular signal-regulated kinase (ERK) activation inhibits apoptosis [25]. Phosphatidylinositol 3-kinase (PI 3-K) is another kinase that can control cell survival by the phosphorylation of Akt in several cell types, including hematopoietic cells [26]. However, it is unclear how intracellular signaling pathways regulate Fas-mediated neutrophil apoptosis in surgical stress-induced inflammation.

Thus, the purpose of this study was to elucidate the effect of the surgery-induced inflammation on Fas-mediated apoptosis of circulating neutrophils. In the present study, the Fas-induced neutrophil apoptosis was inhibited by the presence of autogenous plasma in surgically induced acute inflammatory conditions. The anti-apoptotic action of plasma was not affected by the addition of neutralizing antibodies for inflammatory cytokines such as granulocyte-macrophage colony-stimulating factor (GM-CSF), interleukin-6 (IL-6), and IL-8. These results suggest that pro-inflammatory cytokines are not a dominant factor for the anti-apoptotic effect in the plasma at acute inflammatory conditions. Furthermore, the anti-apoptotic effect of plasma on the Fas-mediated neutrophil apoptosis was attenuated by PI 3-K inhibitor and ERK inhibitor. These results suggest that the anti-apoptotic factors of plasma may, in part, depend on the PI 3-K and ERK signal pathways at surgery-induced inflammatory conditions.

MATERIALS AND METHODS

Reagents and Chemicals

RPMI 1640 medium and FBS were purchased from GIBCO-BRL (Gaithersburg, MD). Murine anti-Fas monoclonal antibody (mAb) (CH-11), FITC-conjugated murine anti-Fas mAb (UB2), MEBCYTO apoptosis kit using FITC-conjugated annexin V and propidium iodide (PI), and soluble Fas (sFas) ELISA kit were obtained from MBL (Nagoya, Japan). Control Abs were obtained from PharMingen (San Diego, CA). Cytokine ELISA kits were purchased from R&D Systems (Minneapolis, MN). The ERK inhibitor, PD98059, the p38MAPK inhibitor, SB203580, and the PI 3-K inhibitors, wortmannin and LY294002, were obtained from Calbiochem (San Diego, CA). Anti-Akt, anti-phospho-Akt, anti-ERK, and anti-phospho-ERK were obtained from New England Biolabs (Beverly, MA). Neutralizing anti-cytokine mAbs, including GM-CSF, IL-6, and IL-8, were obtained from ENDOGEN (Woburn, MA). All other chemicals were of analytical grade.

Subjects

Eight patients (mean age, 23.1 $\pm$ 3.2 years old) who had undergone jaw osteotomy with mandibular prognathism were enrolled in this study. Operations were performed in ordinary general anesthesia and a surgical manner, that is, a bilateral sagittal split ramus osteotomy [27]. The operation time was 189 $\pm$ 42 min, and the blood loss was 482 $\pm$ 180 g. Peripheral blood samples were planned to be harvested from patients at 2 days before, and at 1 and 5 days after, surgery. We defined the operation day as day 0, so postoperative 1 day means the day after operation. All subjects had no sign of inflammatory disease before surgery. None of the patients showed any signs of severe systemic complications during the perioperative period. The study protocol was approved by the Human Ethics Review Committee of the Showa University School of Dentistry, and a signed consent form for the purpose of this study was previously obtained from each subject.

Neutrophil Isolation and Culture

Neutrophil suspensions were prepared as previously described [28], with modifications. Briefly, heparinized blood was mixed with 6% dextran in saline at a ratio of 5:1 and was allowed to settle at room temperature for 40 min. The buffy coat was layered onto a Ficoll-Hypaque (Pharmacia Biotech, Uppsala, Sweden) density gradient and was centrifuged for 40 min at 400 $\times$ g at room temperature. Neutrophils were recovered from the pellet of the gradient by the lysis of contaminating erythrocytes in hypotonic solutions. Neu-