Fas-Mediated Apoptosis of Neutrophils in Sera of Patients with Infection

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In the presence of infection, neutropenia is considered to be a marker of poor prognosis; conversely, neutrophilia may not be a determinant of a better prognosis. Since apoptotic neutrophils are compromised functionally, we evaluated the effect of infection on neutrophil apoptosis. The rate of apoptosis was greater for neutrophils isolated from patients with infection than for healthy controls. Escherichia coli did not directly modulate the rate of neutrophil apoptosis. However, sera from infected patients promoted (P < 0.001) neutrophil apoptosis. Interestingly, the sera of patients with different types of infection (gram negative, gram positive, or culture negative) exerted a more or less identical response on neutrophil apoptosis. Sera of infected patients showed a fivefold greater content of FasL compared to controls. Moreover, anti-FasL antibody partly attenuated the infected-serum-induced neutrophil apoptosis. In in vitro studies, E. coli enhanced monocyte FasL expression. Moreover, conditioned media prepared from activated macrophages from control mice showed enhanced apoptosis of human as well as mouse neutrophils. On the contrary, conditioned media prepared from activated macrophages isolated from FasL-deficient mice induced only a mild degree of neutrophil apoptosis. These results suggest that neutrophils in patients with infection undergo apoptosis at an accelerated rate. Infection not only promoted monocyte expression of FasL but also increased FasL content of the serum. Because the functional status of apoptotic cells is compromised, a significant number of neutrophils may not be participating in the body's defense. Since neutrophils play the most important role in innate immunity, their compromised status in the presence of infection may transfer the host defense burden from an innate response to acquired immunity. The present study provides some insight into the lack of correlation between neutrophilia and the outcome of infection.

Neutrophilia is a common finding in patients with infections. Polymorphonuclear cells are considered to be important players in the host defense system and are the first cells to arrive at the site of injury. Their mobility to sites of infection or inflammation is mediated through the emanation of chemotactic substances, such as formylmethionylpeptides, by neutrophils or macrophages at the site of invasion or by activation of serum complement (19). Neutrophils can be produced at a rate of 100 billion cells daily for an average adult (37), indicating a huge defense potential. Therefore, neutrophils have been considered to function as the primary defenders in acute bacterial infections. However, this defense is compromised in patients with neutropenia, leukemia, or various congenital abnormalities affecting neutrophil structure or function (13, 22, 38).

Infection, directly or indirectly through sympathetic stimulation, induces an increase in blood neutrophil count. Since infection also stimulates the bone marrow to release neutrophils, the appearance of immature neutrophils in the peripheral smear has long been considered a marker of infection. Because neutrophils phagocytize and kill bacteria, one may be tempted to presume that an increased number of neutrophils is associated with a better host defense and an improved outcome. However, there is no relationship between increased neutrophil count and outcome of infection. It is possible that increased neutrophil count in the face of infection may be contributed to by functionally compromised neutrophils. Since apoptotic neutrophils have been reported to lose their function (35), we asked whether infection could promote apoptosis of neutrophils.

Mature neutrophils have a relatively short life span (32, 37). They die through a process of apoptosis (23, 27, 28). It has been demonstrated that interleukin-2, gamma interferon, tumor necrosis factor (TNF) alpha, granulocyte-macrophage colony-stimulating factor, granulocyte colony-stimulating factor, and glucocorticoids increase the life span of neutrophils (4–6, 14, 36). On the contrary, nitric oxide generation, both endogenous and exogenous, may accelerate neutrophil apoptosis (8, 29).

Neutropenia and/or neutrophil dysfunction is often associated with certain microbial agents, such as Staphylococcus aureus and Pseudomonas aeruginosa. In addition, neutropenia in the presence of infection is considered to be a poor prognostic marker.

Proapoptotic factors, such as cell surface expression of Fas and FasL, have been reported to play an important role in the initiation of the apoptotic process for the maintenance of cell population in both physiologic and pathologic states (10–12). Upregulation of monocyte FasL expression has been studied by using non particulate stimuli, such as soluble immune complexes, superantigen, phytohemagglutinin, and antibody-mediated cross-linking of monocytes (16, 26). Various investigators
have reported that monocyes can induce apoptosis in by-
ster cells by modulating the expression of FasL and by
the release of soluble FasL (10, 16, 21, 26). Cells expressing Fas
receptors, including neutrophils, eosinophils, monocyes, and
lymphocytes, may be susceptible to apoptosis induced by cross-
linking with soluble FasL (3, 7, 16, 21, 26).

In the present study we evaluated the occurrence of neutro-
phil apoptosis in patients with infection and in uninfect-
sed subjects. In addition, we evaluated the molecular mechan-
ism of infection-induced neutrophil apoptosis.

MATERIALS AND METHODS

Study subjects. Fifteen patients with infections were selected from the general
medical floor at the Long Island Jewish Medical Center, New Hyde Park, N.Y.
All patients had an elevated white blood cell count and predominant neutro-
philia secondary to bacterial infection. Active infection was characterized by the
presence of fever in the preceding 24 h and microbiological or radiological
evidence of infection. Eight of the patients were male and seven were female.
The patients ranged in age from 30 to 90, with a mean age of 69.8 years. Twelve
patients had either gram-negative (Escherichia coli, two patients; P. aeruginosa,
two patients; Proteus mirabilis, one patient; Bacteroides fragilis, one patient) or
gram-positive bacteria (S. aureus, two patients; Staphylococcus epidermidis, one patient;
Streptococcus pneumoniae, two pa-
tients) infections, and three patients were culture negative. Seven of the patients had
radiographic evidence of pneumonia, two had positive urine cultures, one patient had a
wound infection, and five of the patients had positive blood cultures
without a clear focus of infection. The measured white blood cell counts on the
day of sample collection ranged from 13 × 10^9 to 34.2 × 10^9 cells/mm^3 (a normal
white blood cell count is 3.5 × 10^11 to 11 × 10^11 cells/mm^3). Based on the fact that
they required hospital admission to have antibiotics administered intravenously,
all of the patients were considered to have moderate to severe infections. Con-
trols (six subjects) were healthy volunteers from the institution’s staff who were
on no medications and were free of infection. The absence of infection was
confirmed by following the controls for a period of 2 weeks, during which none of the
controls developed a febrile illness and all had a white blood cell count within the normal range.

E. coli. A clinical pathologic isolate of E. coli (BH5, a derivative of strain TN
675; Takeda Chemical Industries Ltd., Osaka, Japan) (9) was seeded in agar
plates and incubated at 37°C overnight. One colony was picked up with a sterile
pipette tip and added to a 50-ml tube containing medium (RPMI 1640 with 4.5 g
of glucose, 1 mM sodium pyruvate, 2 g of NaHCO_3/Liter) and kept at 37°C for 6 h.
Bacteria were killed by being incubated at 70°C for 60 min and then were
osmopsonized. We evaluated the direct effect of bacteria on neutrophils as well as
monocytes.

Neutrophil isolation. Ten milliliters of peripheral blood was collected from
each donor in heparinized tubes. Neutrophils were separated by a standard
technique of density-gradient centrifugation over Ficoll-Hypaque (29).

Collection of sera. Five milliliters of peripheral blood was collected at the time of
admission (before initiation of antibiotic administration) from each patient, and
sera were separated.

Apoptosis studies. To evaluate the occurrence of apoptosis and necrosis in
neutrophils, we used Hoechst (H)-33342 (Molecular Probes, Eugene, Ore.) and
propidium iodide (Sigma, St. Louis, Mo.). H-33342 stains the nuclei of live cells
and identifies apoptotic cells by increased fluorescence, whereas propidium io-
dide stains the necroosed cells pink (30, 31). We observed a significantly greater
rate of apoptosis in neutrophils incubated in media containing serum-free media
or media with less than 10% serum; therefore, we included 10% serum in the
incubation media.

To determine the rate of neutrophil apoptosis in infected patients and healthy
subjects, equal numbers of neutrophils (10^8) harvested either from infected
patients or healthy subjects were incubated in medium (RPMI 1640) containing
10% feta! cell serum (FCS) for 6, 12, and 24 h.

To determine the direct effect of bacteria on neutrophil apoptosis, equal
numbers of neutrophils were incubated in medium (RPMI plus 10% FCS) con-
taining either variable concentrations of E. coli (serotype O127:B8) (10^3 to 5 ×
10^5 cells/ml) or lipopolysaccharide (LPS) (a bacterial endotoxin, Sigma CN#
L3129) (10, 50, and 100 mg/ml) for 6 and 24 h.

To determine the effect of infection-induced soluble factors on neutrophil
apoptosis, we evaluated the effect of sera on apoptosis of neutrophils isolated
from healthy subjects. Equal numbers of neutrophils were incubated in medium
(RPMI) containing 10% sera of patients with either gram-negative, gram-positi-
tive, or culture-negative infections or containing sera of healthy subjects for 24 h.

To confirm the role of FasL in the infection-induced neutrophil apoptosis, we
examined the effect of anti-FasL antibody-neutralized pooled infected-serum-
induced neutrophil apoptosis. Equal numbers of neutrophils were incubated in
media containing either 10% pooled healthy sera or 10% pooled anti-FasL
antibody-neutralized infected sera (10% infected sera were preincubated with
anti-FasL antibody, 1 μg/ml, for 60 min at 37°C) for 24 h.

At the end of the incubation period, cells were stained with H-33342 and
propidium iodide as described previously (30, 31). The percentages of live,
apoptotic, and necrosed cells were recorded in eight random fields by two
observers unaware of the experimental conditions.

DNA isolation and gel electrophoresis. DNA isolation and gel electrophoresis
are a simple technique to confirm the occurrence of apoptosis (30, 31). It
provides morphologic evidence of DNA fragmentation. In apoptosis, activation
of an endogenous DNase (specific for internucleosomal DNA) may trigger
degradation of DNA into multiple integers of 180 to 200 base pairs. To confirm the
occurrence of neutrophil apoptosis in infected patients, equal numbers of neu-
traphils harvested from either infected patients or healthy subjects were lysed in
DNA lysis buffer. DNA was extracted and run on a 1.8% agarose gel and
gel electrophoresed.

Protein extraction and Western blotting. Equal amounts of sera were collected
from either infected patients or healthy subjects and were mixed with lysis buffer
followed by protein measurement using a bichinchoninic acid kit (Pierce, Rockford,
Ill.). The proteins (20 μg/ lane) extracted from sera were separated on a 4 to 20% gradient
glycacylamide gel and probed for FasL by using rabbit polyclonal anti-FasL antibody. Two sets of experiments were performed.

To determine the FasL kinetics for infected patients, 5 ml of peripheral blood
was collected on days 1 (before antibiotic administration), 2, 5, and 10 in two
patients with gram-negative infection. Proteins were extracted from sera and
separated on a 4 to 20% gradient polyacylamide gel and probed for FasL by using
to monoclonal anti-FasL antibody (Pharmingen, San Diego, Calif.).

Northern blotting and identification of mRNA of FasL. Since neutrophils are
reported not to express FasL, the source of FasL, in serum may be from mono-
cyes or lymphocytes. We evaluated the effect of E. coli or S. aureus on monocyte
expression of FasL. Equal numbers of monocytes harvested from healthy sub-
jects were incubated in medium (Dulbecco’s modified Eagle’s medium plus 10% FCS)
containing either buffer or E. coli or S. aureus (5 × 10^5 bacteria/ml) for 6 h.
Total RNA was extracted. A cDNA probe specific for FasL was used for hybrid-
ization after [32P]dCTP labeling by a random-primed method. The membranes
were stripped to remove the hybridized probe and were reprobed with a glycer-
oldehyde-3-phosphate dehydrogenase (GAPDH) probe to ascertain that similar
amounts of RNA were applied to the gel.

Evaluation of the role of FasL in induction of neutrophil apoptosis. To
evaluate the role of FasL in the induction of neutrophil apoptosis, we studied the
effect of monocyte/macrophage secretory products of control (C57BL/6J, stock
no. 000864; Jackson Laboratories, Bar Harbor, Maine) and FasL-
deficient mice (C57BL/6J, stock no. 001201; the background of this strain is statistically
96.9% C57BL6/like; Jackson Laboratories) mice on neutrophil apoptosis. Peritoneal
macrophages were isolated from control mice and FasL-deficient mice as described previously (24). Macrophages were
characterized by uniform labeling with mouse anti-CD14 antibody (Becton
Dickinson, San Jose, Calif.).

To prepare macrophage secretory products (MS), equal numbers of MøC
(macrophages derived from control mice) and MøFLKO (macrophages derived
from FasL-deficient mice) were incubated with either media alone, aggregated
immunoglobulin G (IgG) (100 μg/ml), or E. coli (5 × 10^5 bacteria/ml) for 2 h at
37°C. At the end of the incubation period, supernatants (MøC-MS; MøFLKO-
FLKO-MS; MøC plus IgG-IgG-MS; MøFLKO plus IgG-FLKO-IgG-MS; MøC
plus E. coli; E. coli-MS; MøFLKO plus E. coli-FLKO-E. coli-MS) were collected,
passed through a 0.2-μm-pore-size filter, and stored at −70°C. Then we examined
the effect of the macrophage supernatants derived from control and FasL-
deficient mice on apoptosis of neutrophils isolated from control mice as well as
healthy human subjects.

Statistical analysis. For comparison of mean values between groups, the
unpaired t test was used. To compare values between multiple groups, analysis
of variance was applied and a Newman-Keuls multiple range test was
used to calculate a q value. All values are means ± standard errors of the
means (SEM) except where otherwise indicated. Statistical significance
was defined as P < 0.05.
RESULTS

The fluorescence microscopic evaluation of apoptosis was reproducible. The occurrence of apoptosis was further confirmed by a DNA fragmentation assay. At 0 h, the neutrophils from neither infected patients nor healthy subjects showed morphologic evidence of apoptosis. However, as neutrophils aged in vitro (Fig. 1 and 2), the number of apoptotic neutrophils increased for infected patients as well as for controls (control: 6 h, 0.2% ± 0.2%; 12 h, 0.5% ± 0.2%; 24 h, 1.3% ± 0.7%; 48 h, 3.6% ± 1.2%; patients: 6 h, 4.9% ± 1.1%; 12 h, 16.3% ± 3.3%; 24 h, 44.2% ± 5.2%; 48 h, 59.6% ± 11.9% apoptotic cells/field). Nevertheless, the rate of apoptosis was many times higher for infected patients than for controls for the same time points (Fig. 2). Neutrophils harvested from infected patients showed DNA fragmentation into multiple integers of 180 bp (data not shown).

To determine the direct effect of bacteria on neutrophil apoptosis, we studied the effect of E. coli as well as LPS (bacterial endotoxin) on neutrophils. Neither E. coli nor LPS modulated apoptosis in neutrophils (control, 1.3% ± 0.7%; E. coli, 0.2% ± 0.1%; LPS, 0.4% ± 0.1% neutrophils/field after 24 h).

To evaluate whether it is a soluble factor in the sera of infected patients which is playing a role in the induction of neutrophil apoptosis, we evaluated the effect of sera of infected patients and healthy subjects on neutrophil apoptosis. As shown in Fig. 3, sera of patients with gram-negative infections accelerated neutrophil apoptosis compared to sera of healthy controls as well as FCS. These results suggest that sera have a factor(s) which promotes neutrophil apoptosis. To determine...
whether intensity of this factor differs in patients with gram-negative, gram-positive, or culture-negative infections, we evaluated the sera of these patients on the basis of neutrophil apoptosis. As shown in Fig. 4, sera from infected patients accelerated neutrophil apoptosis compared to control sera. However, the rate of neutrophil apoptosis was greater for neutrophils treated with gram-negative, culture-negative, or pooled sera than that for gram-positive sera. Neutrophils treated with gram-negative and gram-positive sera showed DNA fragmentation in the form of a ladder pattern, further confirming the presence of an apoptotic factor. The sera of infected patients showed a fivefold increase of FasL compared with control sera.

Since monocyte upregulation of FasL has been found to promote bystander lymphocyte apoptosis (24), we evaluated the effect of E. coli on monocyte and neutrophil expression of FasL. Neutrophils did not express FasL under basal or E. coli-treated states (data not shown), whereas E. coli treatment increased monocyte expression of FasL (Fig. 5). Similarly, monocytes treated with S. aureus enhanced expression of FasL (data not shown). In addition, the sera of infected patients showed an abundance of FasL protein compared to the sera of healthy subjects (Fig. 6).

To determine the role of FasL in the induction of neutrophil apoptosis, we evaluated the effect of activated macrophages (as a source of FasL) derived from normal mice and FasL-deficient mice on murine and human neutrophil apoptosis. As shown in Fig. 7, FLKO-IgG-MS induced apoptosis in a lower percentage of murine neutrophils than IgG-MS. Similarly, E. coli-MS triggered apoptosis in a greater percentage of murine neutrophils than FLKO-E. coli-MS. This effect of macrophage supernatants was dose dependent, indicating the presence of an apoptotic factor. The effect of macrophage supernatants on human neutrophils is shown in Fig. 8. IgG-MS induced apoptosis in a greater percentage of human neutrophils than FLKO-IgG-MS.

To confirm the role of FasL, we evaluated the effect of anti-FasL antibody-neutralized infected sera on neutrophil apoptosis. Neutralized infected sera showed only a mild effect on neutrophil apoptosis compared with infected sera. Anti-FasL antibody attenuated the effect of infected sera on apoptosis in neutrophils (control, 3.9% \( \pm \) 0.4%; infected sera, 16.2% \( \pm \) 0.8%; neutralized infected sera, 6.1% \( \pm \) 0.5% apoptotic neutrophils/field; for infected sera, \( P < 0.001 \) compared with control and neutralized sera).

To determine the kinetics of FasL in infected patients, FasL content in serum was measured in two infected patients on days 1, 2, 5, and 10. As shown in Fig. 9, FasL content in serum decreased with time as well as with cure of infection.

![FasL Western Blot](image-url)

**FIG. 5.** Effect of E. coli on monocyte FasL expression. The upper lane shows monocyte mRNA expression of FasL in control and E. coli-treated conditions. The lower lane shows monocyte mRNA expression of GAPDH under identical conditions.

![FasL Control vs Infected](image-url)

**FIG. 6.** Western blots showing FasL content in serum for control and infected patients. Sera of infected patients showed a fivefold increase of FasL compared with control sera.

![FasL Control vs Infected](image-url)

**FIG. 7.** Effect of activated peritoneal macrophage (derived from either normal mice or FasL-deficient mice) supernatants on murine neutrophil apoptosis. Results (means \( \pm \) SEM) are from four sets of experiments, each carried out in triplicate. FLKO-IgG-MS induced apoptosis in a lower percentage of murine neutrophils than IgG-MS. Similarly, E. coli-MS triggered apoptosis in a greater percentage of murine neutrophils than FLKO-E. coli-MS. This effect of macrophage supernatants was dose dependent, indicating the presence of an apoptotic factor. The effect of macrophage supernatants on human neutrophils is shown in Fig. 8. IgG-MS induced apoptosis in a greater percentage of human neutrophils than FLKO-IgG-MS.
The present study demonstrates that neutrophils have an accelerated rate of apoptosis in patients with infection. *E. coli* did not promote neutrophil apoptosis directly; nevertheless, *E. coli* and *S. aureus* enhanced monocyte expression of FasL. Moreover, sera (irrespective of coming from gram-positive or gram-negative patients) promoted neutrophil apoptosis, indicating the presence of a circulating factor in sera. Interestingly, anti-FasL antibody attenuated infected-serum-induced neutrophil apoptosis. Infected patients also showed an increased content in serum of FasL. Conditioned media from activated macrophages derived from FasL-deficient mice induced less apoptosis in human and murine neutrophils than conditioned media of control macrophages. These results suggest that infection enhances neutrophil apoptosis through FasL which may have been released in the sera from microorganism-activated monocytes.

Neutrophils are the most abundant of all leukocytes. The life of a neutrophil is spent in three environments: marrow, blood, and tissues. Proliferation and maturation take place in the marrow over a period of 13 to 15 days (32), after which mature neutrophils enter the blood stream. This period can be shortened during times of stress. Under steady-state conditions, death and clearance in the tissues balance daily production of neutrophils. Neutrophils are programmed to die by apoptosis at the time of differentiation. Although inevitable, the exact timing of neutrophil death is subject to regulation by external factors (4).

Host defense in response to bacterial invasion utilizes both the innate and acquired immune response. Neutrophils appear to be the important mediators of innate immunity. This is exemplified by the occurrence of recurrent infections in patients with neutropenia (38). Moreover, the susceptibility of humans with neutrophil dysfunction to a wide array of microbial infections would also appear to be good evidence of the importance of neutrophils in microbial clearance in the early stage of infection. With this background, it appears that increased apoptosis of the neutrophils during infection may be an important step in the transition of the host response from its initial stage (which relies predominantly on the components of the innate response, such as neutrophils) to its intermediate and later stages (which rely more heavily on the components of acquired immunity, such as monocytes and antibodies). The present study suggests that neutrophilia may not always mean increased numbers of functioning neutrophils. Moreover, the promotion of apoptosis by infection may transfer host dependency from an innate response to an acquired immunity.

In vivo, apoptotic neutrophils are rapidly cleared by neighboring phagocytes (3). This mechanism of cell death and clearance of neutrophils has been postulated to represent an injury-limiting process (3). Death by necrosis would result in widespread release of neutrophil cytoplasmic enzymes and subsequent extensive tissue destruction. Apoptosis is a highly regulated and genetically directed process. It is induced by intracellular cues, such as DNA damage or osmotic stress, and extracellular cues, including growth factor withdrawal, matrix detachment, and direct cytokine-mediated killing (15, 17, 21, 22). Two main pathways, caspase proteases or the mitochondrial pathway, are involved in apoptotic cell death (15, 17). However, at every level the action of proapoptotic molecules is opposed by a set of inhibitors. A number of signals such as interaction of TNF-FasL with its respective cognate receptor, TNF-R–Fas, induce trimerization of the receptors (TNF-R1, Fas, DR3, DR4, DR5, and DR6 all contain an intracellular "death domain"), recruiting adapter proteins such as FADD to the death domain (17, 25). These adapter molecules then recruit and activate caspase 8. In the mitochondrial pathway, in response to apoptotic signals, proapoptotic bcl-2 family members translocate to and alter the permeability of the mitochondrial membrane, cytochrome c release, and the production of reactive oxygen species. Antiapoptotic members of the bcl-2 family...
family, such as bcl-2, reside in the mitochondrial membrane and may counter these effects. Both caspase and mitochondrial pathways are interconnected; i.e., caspase 8 cleaves Bid (of the bcl-2 family) to produce tBid; in turn, tBid in cooperation with Bad (of the Bcl-2 family) can trigger cytochrome c release, inducing the caspase adapter Apaf-1 to activate caspases 9 and 3. Finally, activation of caspase 3 either through cleavage of caspase 8 or through the mitochondrial pathway causes degradation of proteins. In the present study, Fas (APO-1; CD95)-FasL interaction has triggered apoptosis of neutrophils. We believe that the downstream signaling of infection-induced neutrophil apoptosis involves recruitment of the adapter protein, FADD, followed by caspase 8 and caspase 3 activation. Since phagocytosis of microorganisms by neutrophils is associated with the generation of reactive oxygen species, the mitochondrial pathway also seems to be operative. Whether Fas-FasL interaction and generation of reactive oxygen species are interrelated and/or have additive or synergistic effects on neutrophil apoptosis requires future studies. We plan to probe infection-induced neutrophil downstream signaling in future studies.

Human immunodeficiency virus type 1 gp120 envelope protein has been shown to increase FasL expression in monocytes and to enhance the expression of FasL gene transcription via the FasL gene enhancer-promoter region upregulation (26). Upregulation of FasL mRNA and the associated increase in apoptosis of Fas-susceptible targets have been reported previously (2). Interestingly, macrophage-associated FasL has been shown to trigger selective apoptosis of Fas-susceptible CD4 but not CD8 T cells from human immunodeficiency virus-negative patients (1).

Recently, Brown and Savill demonstrated that exposure of monocytes/macrophages to opsonized zymosan induced the release of soluble FasL (3). The conditioned supernatants containing FasL led to Fas-mediated apoptosis of bystander monocytes and FasL-negative neutrophils. Although macrophages phagocytosing latex beads produced soluble FasL, it did not show proapoptotic effects on neutrophils (3). For the occurrence of bystander cell apoptosis, these investigators hypothesized a requirement of additional soluble factors besides soluble FasL (3).

Recently, it was reported that E. coli directly triggers macrophage apoptosis (33). To determine the molecular mechanism of E. coli-induced macrophage apoptosis, in the present study we evaluated the effect of E. coli on monocyte mRNA expression of FasL. Interestingly, E. coli promoted monocyte mRNA expression of FasL. Since activated monocytes have been demonstrated to express Fas, we propose that E. coli-induced monocyte FasL expression and subsequent Fas-FasL cross-linking may trigger autocrine cell death. Neutrophils have also been demonstrated to express Fas. Interaction between infection-induced increase of FasL (released from circulating monocytes) in serum and neutrophil Fas receptors may accelerate neutrophil apoptosis. In the present study, bacteria did not promote apoptosis of neutrophils; however, sera of infected patients enhanced neutrophil apoptosis, indicating the presence of a soluble factor. That factor seems to be FasL.

We conclude that patients with infection showed accelerated neutrophil apoptosis. This effect of infection on neutrophils seems to be mediated through FasL. We propose that microorganisms may be compromising the innate response and may thus make the host more dependent on acquired immunity. Since microorganisms are capable of promoting apoptosis in neutrophils and monocytes, a transfer of the host defense burden from innate to acquired immunity may be a forced rather than a sound strategy on the part of the host defense system and thus may not always be associated with a positive outcome. The present study provides a plausible explanation for the lack of correlation between neutrophilia and the outcome of infection.

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