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Dendritic Cell Modification of Neutrophil Responses to Infection after Burn Injury

Julia Bohannon,* Weihua Cui,† Edward Sherwood,*‡§ and Tracy Toliver-Kinsky†‡

Burn patients are highly susceptible to infections due to increased exposure through wounds and impairments in a number of immune functions. Dendritic cells (DCs) are important in activation of numerous immune responses that are essential for the clearance of infections. We have found that prophylactic treatment of burn-injured mice with the DC growth factor FLT3 ligand (FL) significantly increases resistance to burn wound infections in a DC-dependent manner that is correlated closely with enhanced bacterial clearance. However, as DCs are not typically microbialidal, the mechanisms by which DC modulation enhances bacterial clearance are not known. Due to the rapid response of neutrophils to cutaneous wounds, and the reported interactions between DCs and neutrophils, we investigated the role of neutrophils in FL-mediated resistance to burn wound infection. This was examined both in vivo and in vitro through neutrophil depletion, supplementation of neutrophils, and assessment of neutrophil chemotaxis following FL treatment. To test the involvement of DCs, CD11c-diphtheria toxin receptor transgenic mice were used to deplete DCs during FL treatment. Studies revealed that neutrophils do play a critical role in FL-mediated resistance to a burn wound infection. Additionally, treatment with FL after a burn injury enhances neutrophil-mediated control of bacterial spread, neutrophil migratory capacity, and myeloperoxidase production in a DC-dependent manner. The results of this study provide new insight into immunological mechanisms that can offer protection against infection after burn injury.


Patients with severe burn wounds are highly susceptible to opportunistic infections as a result of the loss of the protective skin barrier and numerous injury-induced immune alterations that impair the ability to control the spread of infection. Opportunistic infections remain the leading cause of death in burn patients, even with advances in antibiotic treatments and patient care (1, 2). Disruption in innate immune responses following a severe burn injury include impairments in the functions of NK cells, neutrophils, and APCs, all of which are crucial for the establishment of a normal immune response to infection (3–7). Understanding burn injury-induced immune impairments and the development of treatments to overcome these impairments are of critical importance in reducing morbidity and mortality among burn patients.

Dendritic cells (DCs) play a critical role in the recognition of infection and subsequent activation of innate and adaptive immune responses. Langerhans cells, DCs in the skin, are important in trafficking infectious Ags from wounds to the lymph nodes, where activation of immune responses occurs (8). We have previously reported that enhancement of DC numbers and functions through treatment with the hematopoietic growth factor FLT3 ligand (FL) leads to significantly enhanced resistance to a lethal *Pseudomonas aeruginosa* burn wound infection in mice. This increased resistance to infection through treatment with FL is associated with a decrease in bacterial spread (9, 10). However, DCs do not play an active role in bacterial killing or clearance. Therefore, FL-induced modifications of DCs must contribute to the control of bacterial spread in an indirect manner through the activation or enhancement of other cells and bactericidal functions.

Neutrophils are among the first responders to a cutaneous injury, where they function by controlling infection through bacterial uptake and killing and production of soluble factors that initiate activation and recruitment of additional neutrophils and other immune cells to the sites of inflammation and infection (11–13). Unlike DCs, neutrophils do not express the receptor for FL, FLT3R, and cannot be modified directly by FL (14). DCs and neutrophils can interact with each other, leading to bidirectional activation through cell–cell interactions and through secretion of activating cytokines (15). Upon interaction with neutrophils, DCs exhibit an upregulation of costimulatory molecules, and neutrophils are capable of assisting DCs in Ag presentation and activation of T cell responses (16–18). Additionally, interactions with DCs increase the expression of activation markers on neutrophils, as well as the secretion of elastase and myeloperoxidase (MPO), and can delay neutrophil apoptosis (19). Cytokines produced by DCs can enhance bacterial uptake by neutrophils (20). Thus, interactions between DCs and neutrophils provide a potential mechanism for enhancement of bacterial clearance through DC modification by FL.

Using a model of *P. aeruginosa* burn wound infection, we tested the hypothesis that FL treatments enhance the ability of DCs to promote neutrophil-mediated clearance of infection. The results of this study support the hypothesis and provide new insight into immunological mechanisms that can offer protection against infection after burn injury, as well as increase understanding of the role played by interactions between DCs and neutrophils in the immune response to infection.
Materials and Methods

Mice

All animal procedures were consistent with the National Institutes of Health guidelines for the care and use of experimental animals and were approved by the Institutional Animal Care and Use Committee at the University of Texas Medical Branch (Galveston, TX). A full-thickness scald burn was induced as previously described (9). Briefly, male BALB/c mice, 6–8 wk of age, were given buprenorphine (0.1 mg/kg) 30 min prior to burn injury for pre-emptive analgesia, then anesthetized with 2.5% isoflurane, and shaved with clippers on the dorsal and lateral surfaces. Mice were placed on their backs and secured in a protective template with an opening corresponding to 35% of the total body surface area. The exposed skin was immersed for 10 s in 97°C water. Lactated Ringer’s solution (LR; 2 ml) was injected i.p. immediately after burn injury for fluid resuscitation. This volume is a slight increase over that suggested by the modified Brooke’s equation (1.75 ml). Sham-injured mice were handled identically except for immersion in water. FL treatments (10 μg in 0.1 ml LR) were administered once daily by i.p. injection. Control-treated mice received i.p. injections of the same volume of LR solution alone. Recombinant human FL was provided by Amgen (Thousand Oaks, CA). Unless otherwise noted, treatments were started immediately after burn injury. We have found that FL can be started as early as the day of injury or as late as the day of inoculation and provide a similar level of protection against wound infection (10).

Bacterial infection

P. aeruginosa was used because it is a common source of wound infections and pneumonia in burn patients. The culture was obtained from American Type Culture Collection (Manassas, VA; ATCC #19660), and grown in tryptic soy broth, and diluted in sterile saline solution prior to wound inoculation. Unless otherwise noted, mice were inoculated by topical application of a dose that induced ~80% mortality; specifically, 8 × 10^3 CFU applied to the surface of the burn wound at 3 d postburn or 10^4 CFU if applied 4 d postburn. Wounds were inoculated 4 d postburn to mimic the clinical scenario in which patients typically develop infections several days to 2 wk after the initial burn injury (21). This model represents a progressive wound infection that gradually develops by growing within the wound and spreading into the surrounding tissue. Approximately 3 d postinoculation, mice begin displaying symptoms of systemic infection, including positive blood and organ cultures and systemic IL-6, and treatment-associated differences in overall appearance and survival in response to infection become apparent (9, 10). To examine bacterial burden, serial dilutions of tissue homogenates were grown on tryptic soy agar overnight to determine CFU per gram of tissue. MPO was measured by homogenizing in 3 ml lysis buffer (200 mM NaCl, 5 mM EDTA, 10 mM Tris, 10% glycerol, 1 mM PMSF) per gram of tissue. MPO measurements were performed to show that neutrophils were present in wounds and that they were the major source of oxidants. MPO was measured by ELISA according to manufacturer’s directions (Calbiochem, Spring Valley, CA). For immunohistochemistry, wound-draining lymph nodes were fixed in 4% paraformaldehyde and analyzed by an FACSscan flow cytometer (BD Biosciences, San Jose, CA). Staining was determined by comparison with appropriate Ab isotype controls using FlowJo software (Tree Star, Ashland, OR). Fluorescence-conjugated Abs were purchased from Caltag Laboratories (Carlsbad, CA) and eBioscience (San Diego, CA).

Neutrophil and DC depletion

To determine the role of neutrophils in natural and FL-mediated resistance to burn wound infections, neutrophils were depleted by i.p. injection of 150 μg 1A8 clone of the anti-Gr1/Ly6G Ab. Specifically, mice were treated with FL or LR for 4 d beginning on the day of injury, and wounds were inoculated 4 d postburn. Anti-Ly6G Abs were injected prior to wound infection and did not cross-react with Ly6C, as does the RB6-8C5 clone of Gr1 Ab (27). We have confirmed by flow cytometry that the 1A8 clone depletes neutrophils but not T cells, B cells, monocytes, or DCs (not shown). The commercially available preparation contains azide, which was removed using Zeba Desalt Spin Columns (Thermo Fisher Scientific, Rockford, IL) per manufacturer’s directions.

For transient depletion of total DCs at the start of FL treatment, C11c-diphtheria toxin receptor (DTR) transgenic mice [C.FVB-Tg(Itgax-DTR/ GPP57)Jan/J; The Jackson Laboratory, Bar Harbor, ME] were injected i.p. with 4 ng/g diphtheria toxin (DT; Sigma-Aldrich, St. Louis, MO) 1 d after burn injury. FL treatments were initiated 4 h later and were administered for 3 d. Wounds were inoculated on the fourth day after burn injury. For depletion of DCs and the end of FL treatments, which started 1 d after burn injury and were administered for 3 d, mice were injected with 4 ng/g DT on the fourth day after burn injury, followed by wound inoculation 4 h later. In this system, DCs are rapidly depleted and begin to gradually recover after 3 to 4 d (28). Depletion of DCs was confirmed by FACS analysis (not shown). For all experiments shown, tissues and cells were harvested from burn-injured mice.

Cell isolations

DCs and neutrophils were isolated from spleens and lymph nodes of burned mice using Miltenyi magnetic separation columns according to manufacturer’s directions (Miltenyi Biotec, Auburn, CA). The use of Miltenyi microbeads to positively select cells has been extensively published, and the size and biodegradable composition of these microbeads is such that they do not activate the cells or affect cell viability. Total DCs were positively selected using CD11c microbeads, and neutrophils were selected using Ly6G (1A8) microbeads. Cell enrichment was confirmed by FACS analysis.

Adoptive transfers

A total of 4 × 10^6 Ly6G-positive neutrophils, harvested from spleens of donor mice, were injected i.p. into recipient mice immediately prior to wound inoculation. We have previously confirmed successful transfer of CFSE-labeled cells after i.p. injection in the spleen and blood of mice, and others have reported, following direct comparison of adoptive transfer by i.p. and i.v. injection, no differences in trafficking patterns or levels of tissue reconstitution between these two methods (9, 29).

Neutrophil chemotaxis

For in vitro chemotaxis studies, 10^6 cells were plated in the upper chambers of 24-well transwell filter plates with a filter pore size of 3 μm. For stimulation of chemotaxis, 600 μl 2%–diluted cell-free supernatants from spleenocyte cultures that had been stimulated overnight with 100 ng/ml LPS were placed in the lower wells, and cells were incubated at 37°C for 3 h. A total of 7 mM EDTA was added to the bottom wells for 10 min to release any adhered cells from the well and filter. Cells from the lower chambers were then stained with trypan blue and counted on a hemocytometer. For cocultures, DCs were isolated and pretreated overnight with FL (50 ng/ml), then washed to remove FL, and were cocultured with neutrophils (10:1 neutrophils/DCs) overnight. Cells from cocultures were plated in upper chambers for chemotaxis. Total numbers of migrated cells were counted, then stained with PE-conjugated anti-Ly6G and FITC-conjugated anti-CD11c for analysis by FACS to confirm neutrophil numbers. DC chemotaxis was negligible (<1%). All tissues and cells used came from burned mice.

MPO measurements

For quantitative measurement, lymph nodes were harvested 3 d postinfection and homogenized in 3 ml lysis buffer (200 mM NaCl, 5 mM EDTA, 10 mM Tris, 10% glycerol, 1 mM PMSF) per gram of tissue. MPO was measured by ELISA according to manufacturer’s directions (Calbiochem, Spring Valley, CA). For immunohistochemistry, wound-draining lymph nodes were fixed in 4% formaldehyde in Streck fixative, processed to paraffin, and cut at 4 μm. Sections were blocked with nonimmune serum, then incubated with Abs against MPO followed by secondary Abs. Binding was visualized using diaminobenzidine chromagen.

Statistics

GraphPad Prism version 4.0 for Windows was used for all statistical analyses (GraphPad, San Diego, CA). Two group comparisons were made with an unpaired, two-tailed Student t test. Multiple group comparisons were performed using a one-way ANOVA and Tukey-Kramer multiple comparisons test. Survival curves were analyzed by log-rank test.

Results

Both DCs and neutrophils are necessary for resistance to a burn wound infection

We previously reported that FL increases resistance to a burn wound infection and that resistance can be conferred to nontreated
mice by injection of DCs from FL-treated donors. However, injection of NK cells, also increased by FL treatment, and all other leukocytes (non-DC, non-NK) from FL-treated mice did not confer resistance to infection, indicating that FL-modified DCs can mediate the protective effects of FL (9). We confirmed the role of DCs in FL-mediated resistance to wound infection by using CD11c-DTR mice that can be transiently depleted of DCs by injection of DT. Fig. 1A shows that FL significantly increased survival upon wound infection compared with control LR treatment. However, in mice depleted of DCs, either at the start of treatment or several days later at the time of infection, FL was no longer protective. We examined bacterial levels in the spleen as an indication of systemic dissemination of bacteria from the wound 2 d postinoculation. Only three of six FL-treated mice had positive cultures, and bacterial levels in those were negligible, whereas all of the LR-treated mice had positive cultures with substantial numbers of bacteria. Similarly, all of the FL-treated mice that were depleted of DCs at the start of or after FL treatment had positive cultures (Fig. 1B). This supports our earlier reports that FL increases resistance in a DC-dependent manner, but raised the question as to the mechanism of protection, because the predominant function of DCs is to activate other immune cells to eliminate infection.

Earlier studies implicated innate responses in FL-mediated clearance of burn wound infection (30). We examined the contribution of neutrophils to FL-induced resistance to wound infection by injecting mice with Abs against Ly6G to deplete FL-treated mice of neutrophils on the day of wound inoculation. Again, FL significantly increased survival compared with LR treatment, but not when neutrophils were depleted at the time of infection (Fig. 2A). Neutrophil depletion in burned mice did not induce mortality in the absence of a wound inoculation (not shown).

Furthermore, when burned mice were injected with neutrophils from FL-treated mice on the day of wound infection, survival was increased from 50% in mice that received neutrophils from LR-treated donors to 90% in mice that received neutrophils from FL-treated donor mice (Fig. 2B). This finding suggests that FL enhances neutrophil-mediated clearance of a lethal burn wound infection.

**FL treatment enhances neutrophil responses to a burn wound infection**

To examine the effects of FL on neutrophil responses to infection, wound-draining lymph nodes were harvested from FL- or LR-treated mice 3 d following inoculation of burn wounds. Single-cell suspensions were stained with Abs against Ly6G and analyzed by FACS. Following FL treatment, neutrophil numbers were significantly higher (~2 times) in the lymph nodes (Fig. 3A). Fig. 3A also shows representative sections of wound-draining lymph nodes stained for MPO, another neutrophil marker. MPO is not specific to neutrophils, as it can also be produced by monocytes, but the greater staining in FL-treated mice is consistent with the increase in Ly6G cells. Similarly, MPO levels in homogenized lymph nodes were significantly higher following burn wound infection in FL-treated versus LR-treated mice (Fig. 3B).

To eliminate possible treatment-associated differences in local neutrophil chemoattractants, neutrophils were harvested from FL- or LR-treated burned donor mice and injected into nontreated burned mice prior to inoculation of burn wounds as in Fig. 2B. Levels of MPO in wound-draining lymph nodes were measured 3 d later. As shown in Fig. 4A, neutrophil supplementation alone significantly increased MPO levels in wound-draining lymph nodes. However, MPO levels were significantly higher in mice that received neutrophils from FL-treated compared with LR-treated donor mice. Together, these data suggest that FL modifies neutrophils to increase their response to infection.

**Enhanced neutrophil responses to wound infection are DC dependent**

To determine if the effects of FL on neutrophil responses to infection are dependent upon DCs, CD11c-DTR mice were used for transient depletion of DCs during FL treatment as in Fig. 1A. Neutrophils were harvested from donor mice whose DCs had been depleted or left intact during FL treatment and injected into mice as in Fig. 4A. As shown in Fig. 4B, if donor neutrophils were harvested from mice that had been depleted of DCs during in vivo FL treatment, lymph node MPO levels were significantly decreased to levels that were similar to those in control mice that did not receive extra neutrophils. The magnitude of the difference in MPO levels between the no-neutrophil control and the neutrophils from FL-treated mice in Fig. 4B, although less than that shown in Fig. 4A due to natural variability in the live infection model, was statistically significant.

**FL enhances in vitro neutrophil chemotaxis**

To determine if FL enhances the ability of neutrophils to migrate, in vitro chemotaxis was measured. Specifically, neutrophils were isolated from burned mice 4 d postinjury, and chemotaxis across a transwell filter toward cell-free supernatants from LPS-stimulated splenocytes was examined. Significantly more neutrophils (~4.6 times) migrated across the filter when they had been harvested from FL-treated compared with control-treated mice (Fig. 5A). These data indicate that the neutrophils harvested from FL-treated mice had been modified in vivo such that the ability to migrate was enhanced. To eliminate potential effects from circulating mediators that may influence neutrophil responses to FL in vivo, neutrophils were isolated from cultures of total splenocytes that had been previously pretreated in vitro with or without FL. Neutrophils from FL-

![FIGURE 1](http://www.jimmunol.org/) DCs are necessary for FL to promote survival and control of bacterial dissemination from infected burn wounds. Burned mice were treated with FL (n = 8) or LR (n = 5) for 3 d beginning 1 d after burn injury. DCs were depleted by injection of DT 1 d postinjury (DT + FL, n = 12) or 4 d postinjury (FL + DT, n = 5). Survival was monitored for 14 d (A). Spleens were harvested and cultured 3 d after wound inoculation (B). *Significantly different from all others. p < 0.05.
treated cultures also showed significantly enhanced chemotaxis (∼2 times) when compared with neutrophils isolated from control-treated cultures (Fig. 5B).

We confirmed earlier reports that neutrophils do not express the FLT3R (14). FLT3R was not detected on neutrophils, so the effects of FL on neutrophil responses must be mediated via another cell that is FLT3R+, such as DCs (Fig. 6). Because the neutrophils in Fig. 5A and 5B were exposed directly to FL and numerous other cell types in vivo and in splenic cultures, respectively, we examined the ability of DCs to directly modulate neutrophils in vitro. Neutrophils were cocultured with or without DCs that had been isolated from burned mice 4 d postinjury and pretreated overnight in vitro with or without FL. Subsequent in vitro chemotaxis of neutrophils that had been cocultured with control DCs was significantly higher (∼1.9 times) when compared with neutrophils not cocultured with DCs. However, coculture of neutrophils with FL-modified DCs further and significantly enhanced subsequent neutrophil chemotaxis (∼3.2 times) in vitro (Fig. 5C), further suggesting that FL-modified DCs can enhance the ability of neutrophils to migrate.

**Neutrophils from FL-treated mice promote bacterial clearance in a DC-dependent manner**

We have previously reported and confirm in this study (Fig. 1B) that treatment of burned mice with FL reduces systemic dissemination of infection following inoculation of burn wounds (10). Because lymph node MPO levels following i.p. injection of neutrophils were greater when neutrophils had come from FL-treated donors, we sought to determine if bacterial clearance was similarly increased. As shown in Fig. 7, when mice were injected with neutrophils from burned, LR-treated mice, there was no difference in the proportion of mice that had positive spleen cultures 3 d after wound inoculation when compared with control mice that did not receive extra neutrophils. However, when mice received neutrophils from FL-treated mice, only one out of five mice examined had a positive culture compared with four out of five mice that received neutrophils from LR-treated mice or no extra neutrophils at all. Compared to control mice with no neutrophil supplementation, bacterial counts were ∼5 times lower in the group that received neutrophils from FL-treated mice and ∼46 times lower in the group receiving neutrophils from FL-treated mice, although the differences at this sample size were not statistically significant. The same trend was seen in the blood (not shown).

When CD11c-DTR mice were used for a similar experiment, injection of neutrophils harvested from FL-treated donor mice again decreased the number of recipient mice with positive cultures to only one out of five (Fig. 7). However, when DCs were absent during FL treatment of donor mice due to injection of DT at the start of treatment, the number of recipients with positive cultures increased to three out of five, which was the same as in the mice treated with LR alone.
that received no neutrophil supplementation. Bacterial counts were ∼2 times lower in the group receiving neutrophils from DC-depleted FL-treated mice and ∼202 times lower in the group receiving neutrophils from FL-treated mice with DCs remaining intact compared with the vehicle control mice that did not receive any neutrophils.

Discussion

The results of this study provide new insight into immunological mechanisms that can provide protection against a burn wound infection. Earlier data have shown that treatment with FL after burn injury improves survival and bacterial clearance in a DC-dependent manner (9, 10). Although DCs express the FLT3R, these cells do not play a primary role in bacterial killing. Therefore, we sought to determine a means by which FL-mediated modification of DCs can lead to enhanced resistance to a wound infection.

Innate immune cells, especially neutrophils and DCs, play important roles in responses to cutaneous injury and infection. Langerhans cells are involved in trafficking Ags taken up in the skin to the regional lymph nodes for processing and activation of cell-mediated immunity (8). DCs appear to respond to burn injury by migrating to the site of injury, although their specific functions in this context have not yet been identified. Corneal Langerhans cells have been shown to laterally migrate to a local thermal injury (31). Additionally, DCs localize in the dermis at the border of human burn wounds (32). Our studies suggest that FL treatment enhances natural DC responses to burn injury. Neutrophils are among the first local responders to cutaneous injuries and are further mobilized in response to infection (11–13). Substantial influxes of neutrophils from the periphery into the site of cutaneous injury have been detected within 2 d of injury (11). Neutrophils are recruited to the skin by inflammatory mediators and also by chemotactic signals generated by bacteria themselves (33). The primary protective response of neutrophils to infection is to phagocytose and kill invading microorganisms. This study shows that both neutrophils and DCs play a vital role in mediating FL-enhanced survival following a burn wound infection (Fig. 1). Protection afforded by FL following a lethal burn wound infection is completely ablated when neutrophils or DCs are depleted (Figs. 1, 2). However, because neutrophils do not express the FLT3R, their functions cannot be directly affected by FL (Fig. 6). This study suggests that FL interacts with DCs that in turn enhance neutrophil responses to subsequent infection.

The specific cellular mechanisms by which FL-modified DCs promote neutrophil responses in this model of burn wound infection remain to be determined and are currently under investigation. However, other reports of DC–neutrophil interactions suggest some potential mechanisms. Human DCs and neutrophils are able to interact and activate one another through direct cell–cell contact and through production of soluble factors (34, 35). DCs and neutrophils can interact through binding of Mac-1 and carcinoembryonic Ag-related cellular adhesion molecule 1 (CEACAM1) on neutrophils to the C-type lectin DC-specific ICAM-3–grabbing nonintegrin (DCSIGN; CD209) on DCs (36, 37). This binding leads to an increase in the expression of activation markers CD63 and CD64 on neu-
**FIGURE 6.** FLT3R is expressed on DCs but not neutrophils. Single-cell suspensions were obtained from spleens and wound-draining lymph nodes that were harvested from burned mice that had been treated with FL or LR for 4 d. A shows cells that were stained for CD135 (FLT3R) and Ly6G. FLT3R+ cells and Ly6G+ cells are identified in boxes. B shows MHC class II+ cells with high forward scatter, stained for CD135 (FLT3R) and CD11c. Double-positive FLT3R+CD11c+ cells are identified in boxes. The dot plots are representative of staining observed in at least three mice per group in which >95% of the Ly6G-positive cells are FLT3R negative (in both LR and FL groups) and >88% of CD11c-high cells are FLT3R positive in the FL group and 85% in LR. One representative of n = 3 shown.

trophils, an increase in secretion of elastase and MPO, and can also delay neutrophil apoptosis (19). Likewise, upon interaction with neutrophils, DCs show an upregulation of costimulatory molecules CD40 and CD86 (16). Furthermore, neutrophils can present Ag to DCs and assist in DC-mediated activation of T cell responses (17, 18).

This study did not look at the effect that neutrophils may have on DC responses, but did demonstrate that FL modification of DCs appears to enhance neutrophil responses to burn wound infection. The lymph nodes that drain the burn wound have an increased presence of Ly6G- and MPO-positive cells, as well as significantly higher levels of tissue MPO (Fig. 3). Increased levels of MPO following FL treatment are indicative of an enhanced immune response, but the source(s) of tissue MPO was not identified. Because monocytes can also produce MPO, enhanced lymph node levels cannot be attributed to neutrophils exclusively. However, considering that injection of neutrophils into recipient mice increases lymph node MPO, bacterial clearance, and survival, and that neutrophils are necessary for FL-mediated resistance to wound infection, it is likely that the significant effects of FL on MPO levels are due to effects on neutrophils. The enhanced responsiveness of neutrophils to a burn wound infection following FL treatment could be explained by several possible scenarios including enhanced chemotaxis of neutrophils into burn wounds and/or prolonged survival of neutrophils following interaction with FL-modified DCs. Because both Mac-1 and CEACAM1 are involved in regulation of apoptosis in neutrophils, it is likely that binding of DC-SIGN on DCs with either of these could affect neutrophil apoptosis (19, 38). We did not examine the effects of FL on neutrophil survival in this study, but earlier data showed reduced percentages of leukocytes undergoing early stages of apoptosis following treatment with FL after a burn wound infection (10). Therefore, an effect of FL on neutrophil survival cannot be excluded.

It is also possible that FL treatments may increase the migratory capacity of neutrophils through modulation of chemotaxis-associated molecules or chemotactic factors. Both mature and immature human DCs can influence trafficking and recruitment of neutrophils to inflamed tissues through secreted chemokines (39). Additionally, Mac-1 and CEACAM1 receptors on neutrophils are both adhesion molecules that are involved in neutrophil attachment and rolling along the endothelium during chemotaxis. Thus, interactions of DC-SIGN with these receptors could lead to modifications affecting neutrophil migratory capacity (40, 41). Future investigations will focus on the roles of cell–cell contact and secreted factors, and their receptors, on the effects reported in this study and identification of specific DC-induced neutrophil alterations that regulate chemotaxis. Although the specific events directing FL and DC effects on neutrophils have not yet been determined, our data do suggest that FL can mediate enhancement of neutrophil chemotaxis both in vivo and in vitro.

Injection of mice with extra neutrophils increases MPO levels in wound-draining lymph nodes, and this result is further enhanced when neutrophils come from FL-treated mice (Fig. 4). Because these neutrophils were administered to the peritoneal cavity, greater MPO in the wound-draining lymph nodes suggests that the ability of these neutrophils to migrate to infection is enhanced by FL treatment. However, this effect of FL is significantly decreased if DCs are not present during FL treatment of donor mice due to transient depletion (Fig. 4B). These data suggest a DC-dependent modification of the migratory capacity of neutrophils in vivo after FL treatment. This is further supported by the in vitro chemotaxis studies. By removing neutrophils from the animal and into controlled experimental conditions, potential treatment-associated differences in in vivo chemotactic factors were eliminated. Neutrophils harvested from FL-treated mice migrate significantly better in vitro than neutrophils harvested from control-treated mice (Fig. 5A). Additionally, ex vivo treatment of total splenocytes with FL also enhances subsequent chemotaxis of isolated neutrophils (Fig. 5B). Importantly, after coculture with DCs that have been previously treated with or without FL, chemotaxis of neutrophils is significantly greater if DCs were previously exposed to FL (Fig. 5C). Because FL was washed from the DC cultures prior to coculturing, neutrophils were never directly exposed to FL, so the observed alterations are entirely dependent upon DCs. This is the first report that FL can directly interact with and alter the functions of differentiated DCs. The effects of FL on the production and differentiation of DCs...
through interactions with progenitor cells have been well characterized, but the effects that FL has on FLT3IR+ DCs are not known. Our data strongly suggest that FL directly modifies DCs, which in turn can enhance the ability of neutrophils to migrate to infection or a chemotactic stimulus.

The described effects of FL on neutrophils are associated with a positive functional outcome in mice with infected burn wounds. Enhanced MPO in wound-draining lymph nodes following i.p. injection of neutrophils from FL-treated donor mice was associated with an apparent decrease in systemic dissemination of bacteria. From the burn wound and an increase in survival (Figs. 2, 7). Therefore, enhancement of neutrophil-mediated clearance of infection by pharmacological modulation of DCs provides a novel approach for increasing resistance to infections after burn injury.

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Disclosures
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