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Elimination of Senescent Neutrophils by TNF-Related Apoptosis-Inducing Ligand[1]

Julian J. Lum,* Gary Bren,* Rebecca McClure,† and Andrew D. Badley[2†‡]

Neutrophils are phagocytic effectors which are produced in the bone marrow and released into the circulation. Thereafter, they are either recruited to sites of inflammation or rapidly become senescent, return to the bone marrow, and undergo apoptosis. Stromal cell-derived factor 1 (SDF-1) coordinates the return of senescent neutrophils to the bone marrow by interacting with CXCR4 that is preferentially expressed on senescent neutrophils. We demonstrate that CXCR4 ligation by SDF-1 or other CXCR4 agonists significantly increases the expression of both TNF-related apoptosis-inducing ligand (TRAIL) and of the death-inducing TRAIL receptors on neutrophils, which confers an acquired sensitivity to TRAIL-mediated death and results in TRAIL-dependent apoptosis. In vivo administration of TRAIL antagonists results in neutrophilic accumulation within the bone marrow and a reduction in neutrophil apoptosis; conversely recombinant TRAIL administration reduced neutrophil number within bone marrow. Thus, SDF-1 ligation of CXCR4 causes the parallel processes of chemotaxis and enhanced TRAIL and TRAIL death receptor expression, resulting in apoptosis of senescent neutrophils upon their return to the bone marrow. ***The Journal of Immunology,*** **2005, 175: 1232–1238.**

Neutrophils are terminally differentiated effectors whose principal function is to migrate toward sites of inflammation where they exert inflammatory effects through production and secretion of proteases, reactive oxygen species, and other proinflammatory mediators. Neutrophils are produced primarily within the bone marrow and are constitutively released into circulation, yet the constitutive release can be augmented in response to a variety of chemotactins produced at sites of inflammation. Such chemotactins produced at sites of inflammation include TNF-α, leukotriene B_4, C5a, and some chemokines. These circulating neutrophils have two possible fates: migration to tissue sites of inflammation or senescence.

Neutrophils that migrate to sites of tissue inflammation have a prolonged life span and become resistant to both Fas- and TNF-induced apoptosis (1–3). Possible mechanisms which may account for apoptosis resistance of tissue neutrophils are the direct anti-apoptotic effects of GM-CSF (4), complement component C5a, LPS (5), and type 1 IFNs (6), which appear to be dependent on P13K, NF-κB, and protein synthesis (7). In addition, the process of neutrophil transepithelial migration down-regulates proapoptotic regulators, including Fas ligand (FasL) and select caspases (8). At sites of tissue inflammation, neutrophils eventually become apoptotic and are ultimately phagocytosed by macrophages (9). It has been proposed that apoptosis of such tissue neutrophils occurs as a consequence of their phagocytosis of bacteria and bacterial products (10) due to altered transcriptional regulation of proapoptotic genes (11) and/or the direct proapoptotic effects of reactive oxygen species which are produced during the phagocytic process (12). The fate of neutrophils, which do not enter tissues to become part of an inflammatory nidus, is senescence following a short half-life of 6 h. These senescent neutrophils begin to express CXCR4 and become responsive to the chemotactic effects of stromal cell-derived factor 1 (SDF-1)[3] (13). Consequently, senescent neutrophils migrate to areas of high SDF-1 production, including the bone marrow (14). Upon return to the bone marrow, the fate of senescent neutrophils is apoptotic death, although the molecular regulation of senescent neutrophil apoptosis in the bone marrow is unknown.

In different contexts, neutrophils are sensitive to the proapoptotic stimuli FasL (15), TNF (16), and TNF-related apoptosis-inducing ligand (TRAIL) (17). However, it is unlikely that TNF or FasL are involved in apoptosis of senescent neutrophils, since TNF paradoxically inhibits apoptosis of neutrophils cultured for >12 h in vitro (16), and FasL induces chemotaxis and fails to mobilize calcium in aged neutrophils (18, 19). Conversely, fresh neutrophils are TRAIL resistant, yet become sensitive as they age (17), raising the possibility that TRAIL may be involved in senescent neutrophil death.

The interaction between neutrophils and chemokines is becoming defined. In the case of CXCR4 and SDF-1, CXCR4 expression is low on fresh neutrophils, yet increases as neutrophils age (20). Neutrophils that express low to undetectable levels of CXCR4 are consequently SDF-1 unresponsive (21). Senescent neutrophils however express high levels of CXCR4 and become responsive to the chemotactic effect of SDF-1 (13). These senescent neutrophils preferentially home to the bone marrow in a CXCR4-dependent manner where they are eliminated by an as yet undefined apoptosis pathway.

Given the critical importance of time to both the responsiveness of neutrophils toward SDF-1-induced chemotaxis and susceptibility toward TRAIL-induced death, we questioned whether these

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[3] Abbreviations used in this paper: SDF-1, stromal cell-derived factor 1; TRAIL, TNF-related apoptosis-inducing ligand; FasL, Fas ligand; Lz, leucine zipper.

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two processes might be coordinated. Moreover, since we have previously observed that treatment of T cells with the CXCR4 ligand HIV gp120 can induce an acquired sensitivity to TRAIL-mediated killing (42), we questioned whether SDF-1 or other CXCR4 ligands might alter TRAIL and TRAIL receptor regulation in such a way that TRAIL might mediate senescent neutrophil apoptosis. Such speculations are supported by recent observations that TRAIL can limit the neutrophilic response to TNF (22), that TRAIL blockade exacerbates inflammation seen in animal models of type 1 diabetes (23), autoimmune encephalomyelitis (24), and autoimmune arthritis (25), and finally that TRAIL$^{-/-}$ mice have enhanced susceptibility to autoimmune phenomenon (26).

Materials and Methods

Cell lines and reagents

Jurkat T cells were obtained from American Type Culture Collection and maintained in RPMI 1640 supplemented with 10% heat-inactivated FBS, 2 mM L-glutamine, and 100 U/ml each of penicillin and streptomycin (Canadian Life Technologies Products). PBLs from healthy donors were obtained following informed consent, and the protocol was reviewed and approved by the Mayo Clinic Institutional Review Board. PBMCs were depleted of monocytes by plastic adherence for 16–18 h (in 10% human serum from donors with blood type AB) and cultured in RPMI 1640 and 10% AB serum.

Cell treatments

Cells were seeded in a 96-well round-bottom plate (Costar) and incubated for 16 h with a range of doses of monomeric recombinant gp120 IIIb (Immunodiagnostics) or recombinant SDF-1 (R&D Systems), anti-CD4 (Leu3A), or anti-CXCR4 (12G5) at the indicated concentrations (all Abs from BD Biosciences).

Detection of TRAIL/TRAIL receptors

Briefly, 1×10^5 to 1×10^6 cells were resuspended in PBS containing 10% human AB serum. Cells were stained with the following specific Abs (all from Immunex): TRAIL R1 (M271), TRAIL R2 (M412), TRAIL R3 (M430), TRAIL R4 (M445), and TRAIL. Cells were incubated at 4°C, washed twice in PBS, and stained sequentially with anti-mouse biotinylated IgG1/2 (BD Pharmingen) Ab followed by streptavidin-PE (BD Pharmingen). Isotype Abs were used in all cases as controls. FACS was performed on 30,000 events, and analysis was performed using WinMDI software (http://pingv.salk.edu/software.html).

Isolation of neutrophils

Whole blood from healthy donors was mixed in ACD buffer (3% w/v citric acid, 6% w/v sodium citrate, and 4% (w/v) dextrose in ddH_2O stored at 4°C) in a 5:1 ratio. ACD/blood mixture was overlaid on a 5% dextran/6% NaCl solution and inverted 20 times to ensure adequate mixing. Tubes were left standing at room temperature for 45 min. Supernatants were collected and spun at 1150 rpm for 12 min. Pellets were resuspended in 12 ml of ice-cold ddH_2O and 4 ml of 0.6 M KCl to lyse erythrocytes. The mixture was diluted to 50 ml with PBS and spun at 1300 rpm for 6 min and repeated until no erythrocytes remained. Final cell pellets were resuspended in 2.5 ml of PBS, overlaid on 3 ml of Ficoll-Hypaque (Amersham Pharmacia), and spun for 30 min at 1500 rpm. Supernatants were gently removed using a transfer pipette, and pellets containing neutrophils were resuspended in 2 ml of HBSS (Canadian Life Technologies). Flow cytometry was used to verify purity of neutrophils using anti-CD16-PE or isotype control and was typically ≥90% pure.

Chemotaxis assays

A total of 2×10^6 freshly isolated neutrophils was aged for 16 h and then placed on the upper chamber of a chemotaxis plate (NeuroProbe) with medium containing either vehicle control, 100 ng/ml gp120, 5–10 ng/ml SDF-1, or 0.1 μM fMLP in the lower chamber. Chemotaxis was allowed to proceed at 37°C in a 5% CO_2 humidified incubator. At the end of the assay, cells were collected from both upper and lower chambers and viability was assessed by trypan blue staining.

Mouse studies

Fourteen-day-old C57BL6 mice were allowed to acclimate for 2 wk and given standard chow and water ad libitum. Thereafter, mice received 10 μg of antagonistic anti-mouse TRAIL Ab (clone N2B2; BD Pharmingen) or isotype control Ab via tail vein injection. Eighteen hours later, mice were euthanized via CO_2 asphyxiation and blood samples and/or femurs were processed. In separate experiments, mice received either 50 μg of superkiller TRAIL (APOTECH) or albumin (control). Forty-eight hours later, these mice were euthanized via CO_2 asphyxiation. Peripheral blood was harvested in EDTA tubes and analyzed by manual differential counts in a blinded manner. Bone marrow aspirates were prepared by cutting femurs lengthwise and teasing the marrow onto microscope slides, mixing with a drop of PBS to create a suspension which was then spread and allowed to air dry. Slides were then stained with Wright-Geimsa stain and analyzed in a blinded fashion. TUNEL staining was performed as previously described (27) on femurs that were decalcified, embedded, and cut into 5-μm sections. This protocol was reviewed and approved by the Mayo Clinic Institutional Animal Care and Use Committee.

Immunohistochemistry

Granulocytic Ly-6G-expressing cells were immunostained with an RB6-8C5 monoclonal antisera. Bone marrow aspirate slides were rinsed with TBS/Tween 20 for 5 min before immunostaining. Endogenous peroxidase was quenched with 0.03% hydrogen peroxide, and samples were treated with diluted rabbit serum before primary Ab addition. Cells were incubated with rat anti-mouse Ly-6G (BD Pharmingen) diluted 1/50 for 60 min. Sections without primary Ab served as negative controls. The Vectastain ABC Elite (Vector Laboratories) system and DAB$^+$ (DakoCytomation) was used to produce localized, visible staining. Slides were lightly counterstained with Mayer's hematoxylin, dehydrated, and coverslipped.

Results

CXCR4 ligation of neutrophils, including the neutrophil chemotactin SDF-1, induces TRAIL sensitivity and alters TRAIL receptor expression

We have previously demonstrated that CXCR4 ligation of T cells by agonistic Ab, or the HIV-encoded CXCR4 ligand gp120 (42) can alter TRAIL receptor expression and TRAIL sensitivity. In this study, we addressed whether such a phenomenon may be of physiologic relevance. In particular, we were interested in evaluating senescent neutrophils, since these cells express CXCR4, are SDF-1 responsive, and become both TRAIL sensitive and apoptotic following their SDF-1 directed return to the bone marrow.

We first assessed whether freshly isolated neutrophils were sensitive or resistant to leucine zipper (Lz) TRAIL, and Lz-FasL. Consistent with previous reports, neutrophils incubated overnight with control medium were sensitive to Lz-FasL (mean percent apoptotic cells = 62.6% (n = 6)), but relatively resistant to Lz-TRAIL (mean percent apoptotic cells = 27.6% (n = 6, p = < 0.05)). Conversely, when neutrophils were coincubated overnight with the chemokine SDF-1, cells remained sensitive to Lz-FasL (mean percent apoptotic cells = 72.6% (n = 5)) and became highly sensitive to Lz-TRAIL (mean percent apoptotic cells = 68.1% (n = 5)). Therefore, treatment of neutrophils with SDF-1 induces an acquired sensitivity to TRAIL-mediated killing in neutrophils.

We next assessed neutrophil expression of TRAIL and TRAIL receptors and how these might be altered by SDF-1. Consistent with previous reports, neutrophils expressed detectable levels of TRAIL and all four TRAIL receptors (17, 28) (Fig. 1*A*). Neutrophils treated overnight with gp120 or SDF-1 significantly (p < 0.02) increased expression of all four TRAIL receptors and ligand when compared with cells treated with vehicle control (n = 3, Fig. 1*A*). Of particular relevance is whether the changes observed in neutrophils rendered them TRAIL sensitive. We therefore pretreated neutrophils with SDF-1, gp120, or vehicle control followed by Lz-TRAIL and found that cells treated with vehicle control were resistant to TRAIL, whereas both gp120 and SDF-1 rendered neutrophils highly sensitive to TRAIL-mediated apoptosis (n = 3,

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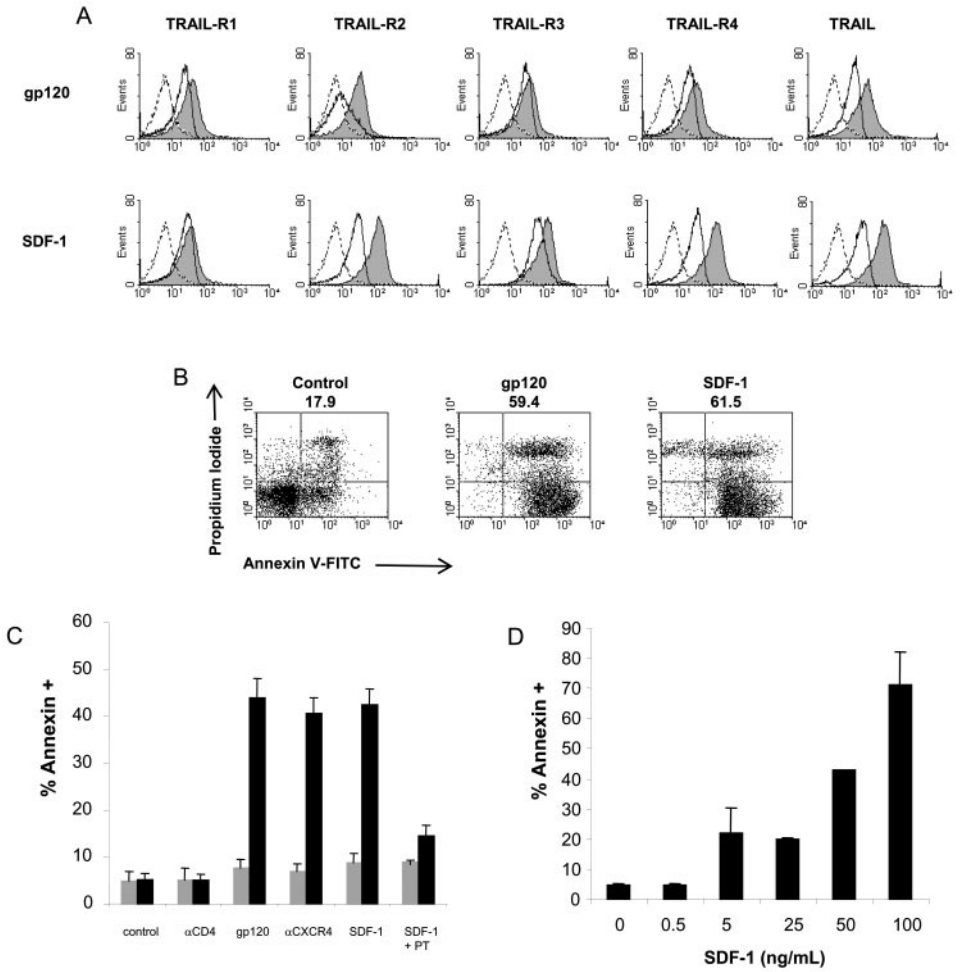

FIGURE 1. Enhanced TRAIL receptor expression and acquired TRAIL sensitivity following CXCR4 ligation. *A,* Expression of TRAIL/TRAIL receptor on neutrophils. Twelve- to 16-h treatment of neutrophils with 100 ng/ml gp120 (*top*) and 100 ng/ml SDF-1 (*bottom*) increase TRAIL/TRAIL receptor expression. Dashed histogram, Isotype control; open histogram, control treatment; shaded histogram, gp120 or SDF-1 treatment (representative of three independent experiments). *B,* Increased cell death in neutrophils by 100 ng/ml gp120 and 100 ng/ml SDF-1 following 24-h treatment. Percentage of apoptosis is denoted on *top* of the dot plots and was counted by annexin V-positive/propidium iodide-negative cells. Representative data from three independent experiments. *C,* Primary neutrophils from healthy donors were stimulated with 1 μg/ml anti-CD4, 100 ng/ml gp120, 1 μg/ml anti-CXCR4, or 50 ng/ml SDF-1 for 24 h, then incubated with Lz-TRAIL. Cell death was assessed by annexin V staining and the average of five experiments was plotted. Gray bars indicate Lz-TRAIL treatment alone and black bars indicate pretreatment with the indicated stimulus followed by Lz-TRAIL. *D,* Dose response of SDF-1 on neutrophil sensitivity to Lz-TRAIL. Neutrophils were treated with increasing concentrations of SDF-1 followed by Lz-TRAIL as in *C*. Cell death was assessed by annexin V staining and an average of three independent experiments was plotted.

$p < 0.01$; Fig. 1*B*). The effects of gp120 (data not shown) and SDF-1 on TRAIL sensitivity was dose dependent (Fig. 1*D*). In contrast, treatment with agonistic anti-CD4 (Fig. 1*C*) failed to induce TRAIL sensitivity, whereas agonistic anti-CXCR4 Ab or the CXCR4 ligands gp120 or SDF-1 all rendered neutrophils TRAIL sensitive ($p < 0.02$ compared with control), suggesting that CXCR4 receptor ligation mediates this effect. Indeed, inhibition of SDF-1-induced TRAIL sensitivity by the G-coupled protein inhibitor pertussis toxin supports this view (Fig. 1*C*).

SDF-1 initiated neutrophil chemotaxis, altered TRAIL/TRAIL receptor expression and cell death

Previous publications suggest that SDF-1 induces neutrophil chemotaxis (13), and our data suggest that SDF-1 also induces enhanced TRAIL receptor expression, an acquired sensitivity to TRAIL-mediated killing, and up-regulation of TRAIL. Altogether, these findings, coupled with high production of SDF-1 from the bone marrow, suggest the possibility that SDF-1 stimulates the return of senescent neutrophils to the bone marrow, followed by initiation of a TRAIL-mediated apoptotic death program within the bone marrow.

To assess this possibility, we first measured the chemotactic effect of SDF-1 on neutrophils aged for 16–18 h and assessed the proportion of migrating neutrophils that die following chemotaxis. Aged neutrophils (>95% viable) were placed in the upper chamber of a chemotaxis plate and either control medium, or medium supplemented with gp120, SDF-1, or the bacterial chemotactin fMLP was added to the lower chamber. Control medium and gp120-treated medium caused a similar but low degree of chemotaxis and death (Table I). Both the bacterial positive control peptide (fMLP) and SDF-1 caused significant chemotaxis. However, only SDF-1 increased spontaneous death to 47.5% of migrating neutrophils ($n = 3$; $p = 0.03$).

Since in primary neutrophils, chemokine receptor ligation with gp120, agonistic Ab, or SDF-1 induces an acquired sensitivity to TRAIL-mediated killing and enhances TRAIL expression, it is

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Table I. *Chemotaxis assay of aged neutrophils*[a]

Treatment	No. of Input Neutrophils (upper chamber)	No. of Neutrophils Recovered in Lower Chamber	% Chemotaxis	% Viability of Neutrophils in Lower Chamber
Control	2×10^6	6.02×10^5	30.1	72.67
gp120	2×10^6	6.88×10^5	34.4	85.17
fMLP	2×10^6	11.22×10^5	56.1	81.11
SDF-1	2×10^6	18.90×10^5	94.5*	52.49*

[a] Neutrophils were seeded in the upper chamber. Ten nanograms per milliliter, SDF-1, 50 ng/ml gp120, 0.1 μM fMLP, or vehicle control was added to the lower chamber and incubated for 18 h. The number of migrated cells and viability was assessed by trypan blue exclusion and the mean of three experiments is presented. *, $p < .05$.

possible that the combination of enhanced susceptibility to and the enhanced production of TRAIL together result in autocrine or paracrine TRAIL-mediated apoptosis of SDF-1-treated neutrophils.

To formally test this hypothesis, we treated neutrophils with SDF-1 in the continuous presence of 1 μg/ml inhibitory anti-TRAIL receptor or anti-FasL Abs. Since SDF-1 induced both enhanced TRAIL R1 and R2 expression, Abs to TRAIL R1 and TRAIL R2 were used. On day 1, spontaneous apoptotic neutrophils were detected by flow cytometry in all cases (Fig. 2). On day 3, the number of apoptotic neutrophils in control treatments was not different from cells treated in the presence of anti-TRAIL R2 and anti-FasL. In contrast, in cultures treated with anti-TRAIL R1, death was reduced from 81.9 to 22.7% ($n = 6$, $p < 0.01$; Fig. 2). Anti-TRAIL R1 treatments also reduced gp120-induced death of neutrophils from 60.2 to 20.5% ($n = 6$, $p < 0.03$, data not shown). These experiments demonstrate that TRAIL R1, but not TRAIL R2, is required for neutrophil death signaling following activation with SDF-1 and gp120 and, moreover, suggest that the TRAIL-TRAIL R1 interaction is supplied in a paracrine fashion. Of interest, however, are our observations that both TRAIL R1 and TRAIL R2 are up-regulated by SDF-1, yet only TRAIL R1 inhibition limits the observed death. Such observations support previous speculations that TRAIL R1 regulates apoptosis associated with inflammation.

TRAIL receptor blockade increases neutrophil number in vivo

SDF-1 recruits senescent neutrophils to the bone marrow (13) (and potentially other sites of high SDF-1 expression such as spleen and liver), which then, according to our hypothesis, die in a TRAIL-dependent manner. Therefore, in vivo systemic TRAIL blockade

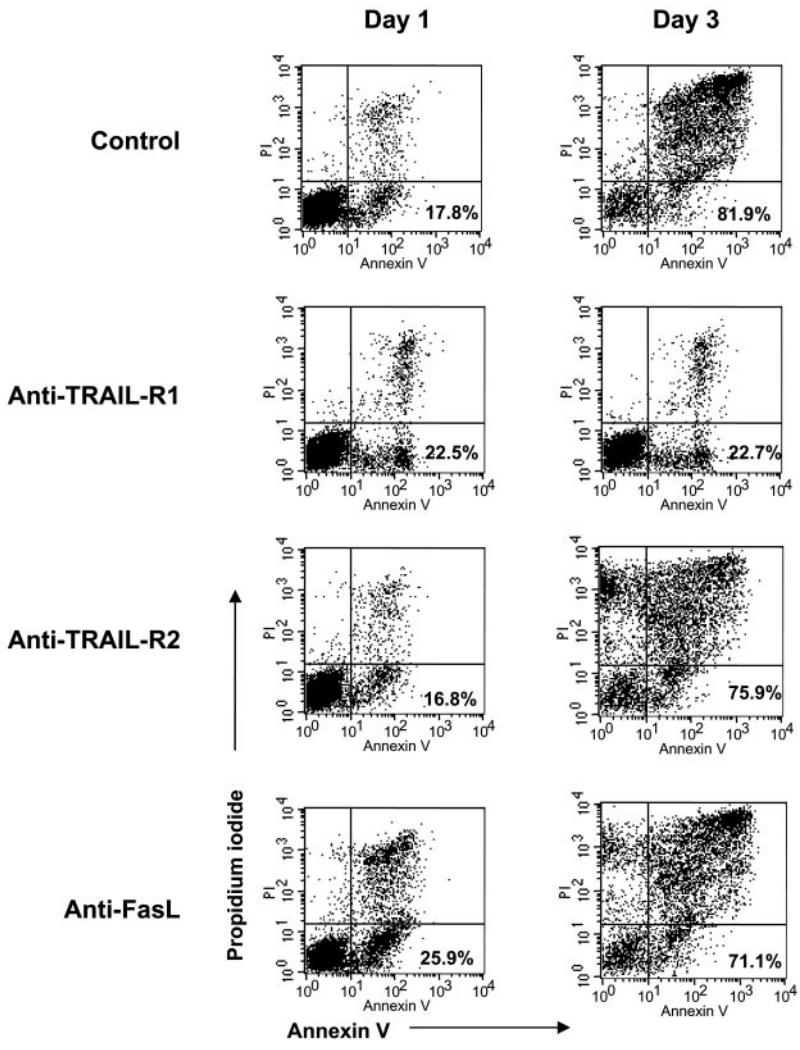

FIGURE 2. TRAIL R1 antagonists inhibit SDF-1-mediated death of neutrophils. Neutrophils were seeded in 96-well plates and activated with 10 ng/ml SDF-1 in the continuous presence of 1 μg/ml TRAIL R1-, R2-, or FasL-neutralizing Abs. On days 1 and 3, cells were collected and stained with annexin V, FITC, and propidium iodide. SDF-1 activation-induced death of neutrophils was blocked by anti-TRAIL R1, but not anti-TRAIL R2 or anti-FasL Abs. Data are representative of six independent experiments and values denote the percent annexin-positive cells.

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should reduce the amount of neutrophil apoptosis that occurs in the bone marrow. We therefore injected mice i.v. with blocking anti-mouse TRAIL Abs or isotype-matched control Abs. Neither Ab caused ill effects in mice, and no pathologic abnormalities were apparent either grossly or histologically when animals were euthanized 16 h after injection. Peripheral blood was evaluated by manual differential enumeration from paired age/sex-matched littermate mice treated with isotype control Ab or an antagonistic anti-TRAIL Ab. Mean neutrophil percent was 8.6% in isotype-treated mice ($n = 18$) and 10.9% in anti-TRAIL-treated mice ($n = 18$, $p = 0.038$, Figs. 3*A* and 4). Bone marrow aspirates were harvested from the femurs of the same control-treated or anti-TRAIL-treated mice and analyzed. No abnormalities within the bone marrow were apparent other than changes in neutrophil number. Geimsa staining revealed subtle increases in neutrophil number that were not statistically significant. Immunostaining with mouse neutrophil-specific Abs (Ly6G) identified increased neutrophil numbers in anti-TRAIL-treated, but not isotype-treated mice. Comparing four random fields per mouse sample, of five mice per group, demonstrated 20.1% immunoreactive cells in the isotype group, yet 33.6% of cells were Ly6G immunoreactive in the anti-TRAIL group (Fig. 3*B*, *top*, $p = 0.01$). Finally, we compared bone marrow biopsies from both treatment groups by TUNEL staining for apoptotic cells. Isotype control-treated samples had 8.1% TUNEL-positive cells, whereas anti-TRAIL-treated samples had 2.3% apoptotic cells (Fig. 3*B*, *bottom*, $p = 0.01$), indicating that anti-TRAIL treatment reduced the number of apoptotic cells in the bone marrow.

TRAIL agonists decrease neutrophil numbers in vivo

Given our findings that TRAIL antagonists increase neutrophil number in vivo, we next performed the corollary experiment. Mice were injected with 10 μg of recombinant TRAIL or an equivalent dose of albumin as control and sacrificed 24 h later. No adverse effects of TRAIL administration were observed in treated mice either clinically or at necroscopy. Consistent with our model that only senescent and SDF-1-responsive neutrophils are TRAIL sensitive, TRAIL injections did not alter neutrophil number in the peripheral blood of mice compared with albumin-treated controls. However, assessment of neutrophil number within bone marrow was altered by TRAIL treatment; Ly6G staining of bone marrow revealed 25.7 $\pm$ 3.32% of bone marrow cells staining with the neutrophil-specific Abs in control mice ($n = 10$); yet 15.72 $\pm$ 1.61% of bone marrow cells stained positive for Ly6G in the TRAIL-treated group ($n = 10$, $p = 0.01$; Fig. 3*C*). In addition, TRAIL-treated mice had greater degrees of TUNEL-positive cells in the bone marrow; isotype-treated mice had 6–8% TUNEL-positive cells, whereas TRAIL-treated mice had 13.7% TUNEL-positive cells ($p < 0.03$).

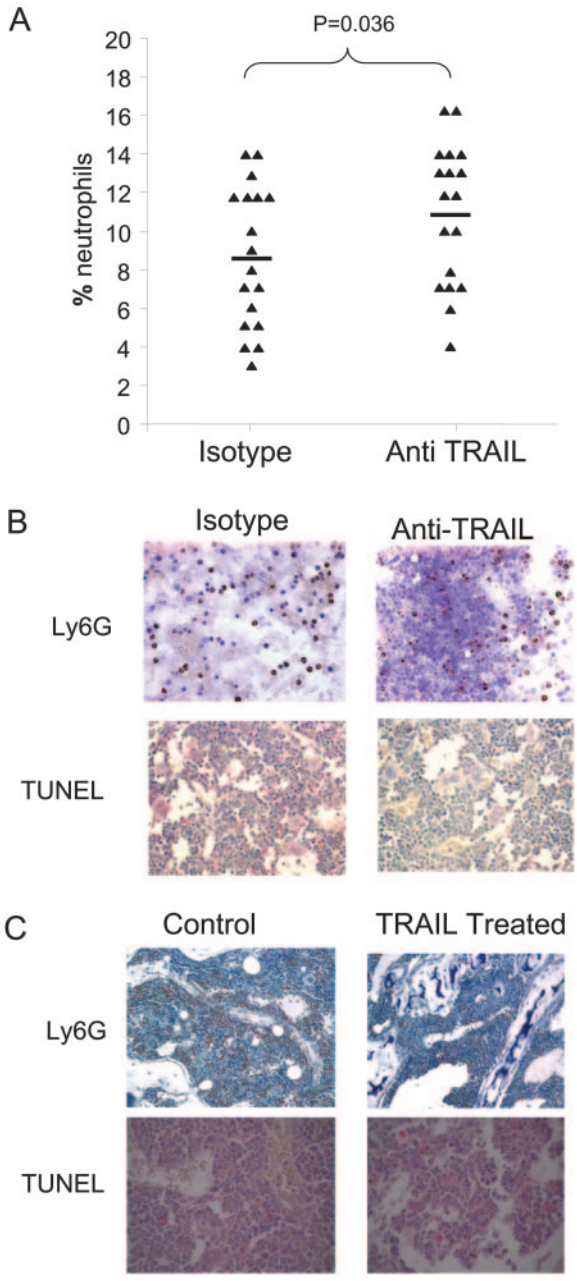

FIGURE 3. Effects of TRAIL agonists and antagonists on neutrophils in mice. Mice were treated with anti-TRAIL Ab or an isotype control and analyzed for peripheral neutrophil number (*A*). *B, Top,* bone marrow aspirates from mice treated with anti-mouse TRAIL Ab or isotype control Ab as indicated and stained with neutrophil-specific Ab LyG6. *Bottom,* Bone marrow biopsies from mice treated as above and stained by TUNEL. *C,* Mice were treated with recombinant TRAIL and bone marrow was analyzed by immunohistochemistry for neutrophil number (*top*) and TUNEL (*bottom*).

Discussion

Neutrophils are polymorphonuclear leukocytes that have a constitutively short life span which is limited by an apoptotic cell death program (2). The molecular regulation of neutrophil apoptosis is unclear, but in different contexts, neutrophils are sensitive to the death receptor-initiated apoptosis induced by TNF (16) and FasL (29). Recently, apoptosis of aged neutrophils have been shown to be accelerated by TRAIL, suggesting a role for TRAIL in eliminating senescent neutrophils (17). However, TRAIL antagonists do not modify spontaneous apoptosis of fresh neutrophils, suggesting that either the involvement of TRAIL in neutrophil apoptosis may be restricted to aged neutrophil apoptosis (17) or that another cofactor is required.

Our study confirms that SDF-1 induces chemotaxis of aged neutrophils and, moreover, we demonstrate for the first time that this stimulus also renders neutrophils TRAIL sensitive. Furthermore, we show that SDF-1 treatment of neutrophils alters the TRAIL/TRAIL receptor expression profile and initiates a paracrine TRAIL:TRAIL receptor-dependent death program. Thus, the acquisition of high CXCR4 expression by aged neutrophils allows responsiveness to SDF-1, which achieves two goals: chemotaxis to the bone marrow where they are destroyed and acquisition of

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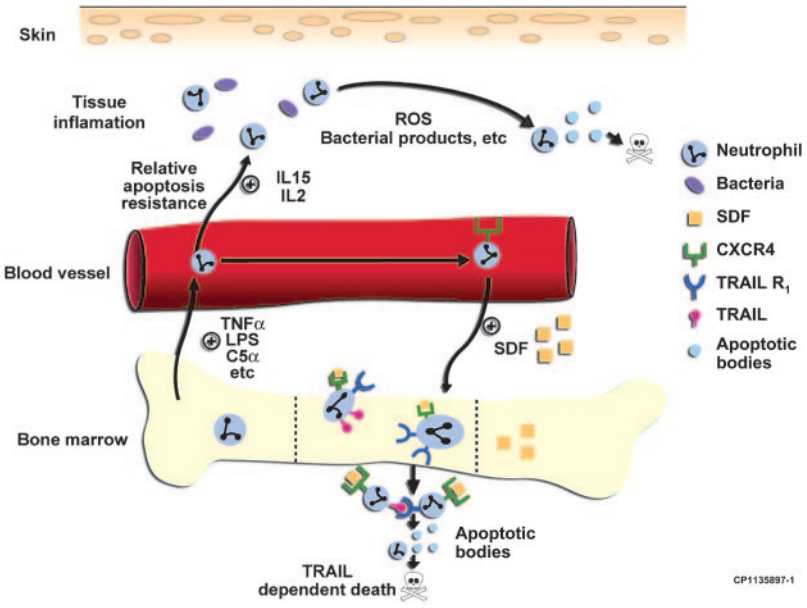

FIGURE 4. Model of neutrophil chemotaxis and survival. Schematic representation of the determinants of neutrophil survival. Immature neutrophils (*bottom left*) are recruited into the circulation by a variety of mediators where they can either enter tissues in response to IL-2, IL-15, and acquire a relative resistance to apoptosis. Thereafter, they die by apoptosis due to the effects of bacteria, bacterial products, or reactive oxygen species (ROS). Once in the circulation, they may age whereupon they acquire CXCR4 and become responsive to the chemotactic effects of SDF-1, which are produced in the bone marrow. SDF-1 also induced TRAIL and TRAIL receptor expression which causes apoptosis of senescent neutrophils.

TRAIL sensitivity and TRAIL production with which they are destroyed. This model is supported by our in vivo results; mice receiving antagonistic anti-TRAIL Abs have inhibited neutrophil apoptosis and neutrophil accumulation within the bone marrow, whereas mice receiving recombinant TRAIL have reduced numbers of bone marrow neutrophils. Moreover, this model reconciles an intriguing observation that although SDF-1 is a potent chemotactin for neutrophils in vitro, s.c. administration of SDF-1 in mice fails to recruit neutrophils. We propose that this is due to all SDF-1-responsive neutrophils having already migrated to the bone marrow where, due to the impact of SDF-1 on TRAIL and TRAIL receptors, the neutrophils will have been eliminated. Indeed, when we s.c. injected C57BL/6-c mice with up to 10 μg of recombinant mouse SDF-1, we failed to elicit either a visible or microscopic neutrophilic inflammatory response (data not shown).

The molecular regulation of TRAIL sensitivity is impacted by several factors. Among these is the relative expression of death receptors (TRAIL R1 and R2) vs decoy receptors (TRAIL R3, and R4) and osteoprotegerin (30–34). However, reasons why multiple TRAIL receptors exist is unclear, although our data provide support for a model where TRAIL R2 is involved in immune surveillance of transformed or virally infected cells (35), whereas TRAIL R1 is involved in regulation of inflammation and inflammatory disorders (36). In our system, both TRAIL R1 and TRAIL R2 were variably increased by chemokine receptor agonists; however, only TRAIL R1, but not TRAIL R2 antagonists, abrogated senescent neutrophil apoptosis, thus supporting a role for TRAIL R1 in the regulation of inflammation.

Our observations in neutrophils highlight an additional level of regulation of TRAIL sensitivity. In our freshly isolated neutrophils, both TRAIL receptors 1 and 2 are present, yet cells remain TRAIL resistant. Reasons for this seeming paradox are unclear; however, similar observations have been reported by numerous other groups (7, 37, 38). Indeed, it is now apparent that not only must cells express TRAIL death receptors, but also require priming for sensitivity to TRAIL-induced apoptosis. Such priming effects can be associated with down-regulation of FLICE-like inhibitory protein and/or other downstream apoptosis-regulating molecules. In vitro treatment of cancer cells with TRAIL is often coupled with chemotherapy or radiation therapy to enhance TRAIL sensitivity and/or reverse TRAIL resistance (reviewed in Ref. 39).

Considerable indirect evidence also suggests that TRAIL may regulate elements of inflammation; for example, TRAIL/TRAIL receptor dysregulation is seen in the inflammatory conditions of Sjögren's syndrome (36), adenoviral hepatitis (40), and autoimmune thyroiditis (41). Furthermore, chronic TRAIL blockade exacerbates collagen-induced autoimmune arthritis (25) and myelin oligodendrocyte glycoprotein-induced experimental autoimmune encephalomyelitis (24), suggesting that TRAIL may directly reduce those inflammatory responses. Our data support those suggestions and argue for a broader role of TRAIL in regulating inflammatory neutrophil survival. Although our data are specific for aged neutrophils, our data do not exclude a possible role for TRAIL in apoptosis of tissue neutrophils.

The property of neutrophils being able to rapidly mobilize in response to inflammatory stimuli also requires them to be able to reduce their numbers once their functions are complete. Although it is acknowledged that inflammatory mediators (e.g., IL-2, TNF-α, IL-15, LPS, IFN-α) both activate and increase the survival of neutrophils to several days (2), it remains controversial what molecular events govern either tissue neutrophil or circulating senescent neutrophil death. Our data demonstrate that one mechanism by which senescent neutrophils are removed is achieved as a consequence of the same chemotactic signal which recruits senescent neutrophils to the bone marrow. SDF-1 initiates a TRAIL: TRAIL R1-dependent activation-induced cell death program which limits the survival of the same neutrophils it recruits. Importantly, the age-dependent expression of CXCR4 specifies that SDF-1 only recruits and, therefore, only kills aged neutrophils.

Disclosures

The authors have no financial conflict of interest.

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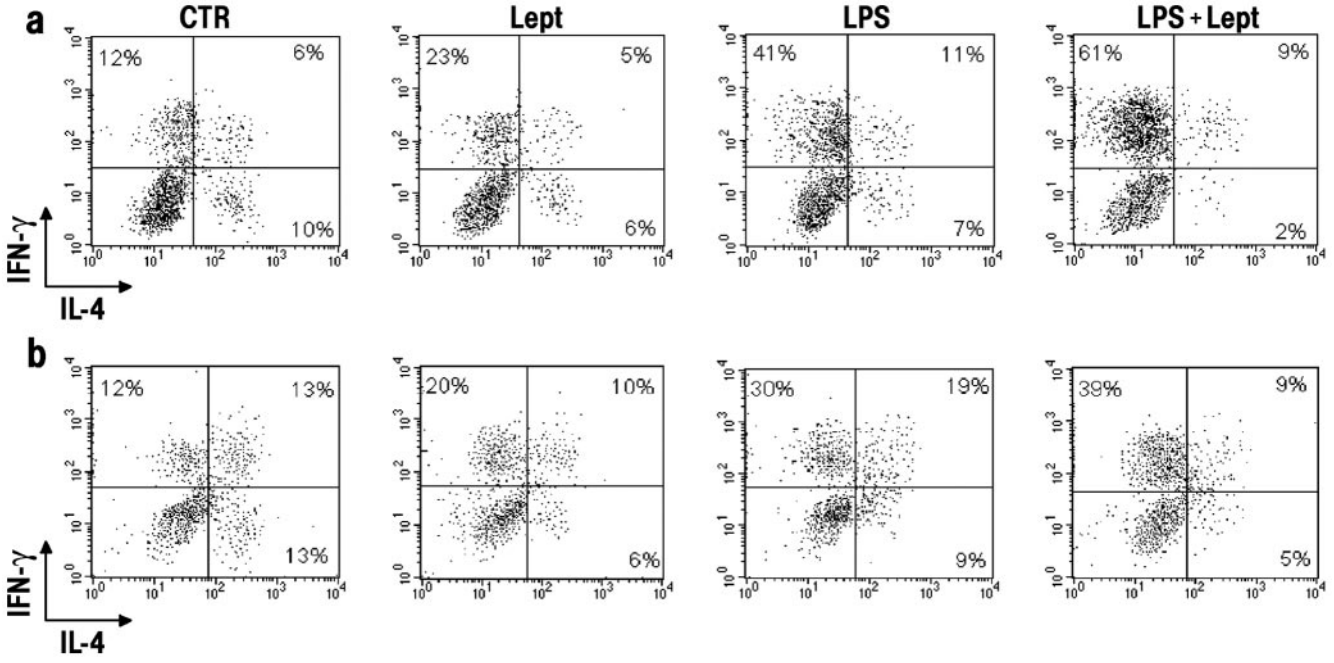
CORRECTIONS

Lum J. J., G. Bren, R. McClure, and A. D. Badley. 2005. Elimination of senescent neutrophils by TNF-related apopotosis inducing ligand. *J. Immunol.* 175: 1232–1238.

The word "apoptosis" was misspelled in the title, in the fourth sentence of the **Abstract**, in the first sentence of the third paragraph of the **Introduction**, and in the abbreviations list in **Footnotes**. The error has been corrected in the online version, which now differs from the print version as originally published.

Mattioli B., E. Straface, M. G. Quaranta, L. Giordani, and M. Viora. 2005. Leptin promotes differentiation and survival of human dendritic cells and licenses them for Th1 priming. *J. Immunol.* 174: 6820–6828.

In **Results,** in Fig. 7, the arrows indicating IL-4 and IFN-γ are inverted. The corrected figure is shown below.



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Li, Z., W. K. Lim, S. P. Mahesh, B. Liu, and R. B. Nussenblatt. 2005. Cutting edge: in vivo blockade of human IL-2 receptor induces expansion of CD56^{bright} regulatory NK cells in patients with active uveitis. *J. Immunol.* 174: 5187–5191.

In **Results and Discussion**, after the first sentence in the last paragraph, the authors wish to add the sentences shown below. In **References**, the authors also wish to add the citation shown below.

Recently, Bielekova et al. described their findings concerning the induction of CD56^{bright} NK cells by anti-IL2R therapy in multiple sclerosis patients (31). This is consistent with our observations in uveitis patients.

31. Bielekova, B., S. Reichert-Scrivner, M. Cerna, H. McFarland, and R. Martin. 2004. CD56^{bright} NK cells mediate immunomodulatory effects of IL-2R-targeted therapy in multiple sclerosis. *J. Neuroimmunology* 154: 211 (Abstr. 699).

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In **Disclosures**, a potential financial conflict of interest was inadvertently omitted. The disclosure shown below should have been declared.

R. A. B. Ezekowitz is cofounder and serves on the Board of Directors of NatImmune, a privately held biotechnology company.

Mc Allister F., C. Steele, M. Zheng, E. Young, J. E. Shellito, L. Marrero, and J. K. Kolls. 2004. T cytotoxic-1 CD8+ T cells are effector cells against pneumocystis in mice. *J. Immunol.* 172: 1132–1138.

The first author's name is listed incorrectly. The correct name is Florencia McAllister.

Chaouat G., A. A. Meliani, J. Martal, R. Raghupathy, J. Elliot, T. Mosmann, and T. G. Wegmann. 1995. IL-10 prevents naturally occurring fetal loss in the CBA x DBA/2 mating combination, and local defect in IL-10 production in this abortion-prone combination is corrected by in vivo injection of IFN-τ. *J. Immunol.* 154: 4261–4268.

The fifth author's name is listed incorrectly. The correct name is John F. Elliott.