Mobilization of Opioid-containing Polymorphonuclear Cells by Hematopoietic Growth Factors and Influence on Inflammatory Pain

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Background: Leukocytes can control inflammatory pain by secretion of opioid peptides, stimulated by cold-water swimming or local injection of corticotropin-releasing factor, and subsequent activation of opioid receptors on peripheral sensory neurons. This study investigated whether mobilization of polymorphonuclear cells (PMN) by granulocyte colony-stimulating factor (G-CSF) and stem cell factor (SCF) enhances immigration of opioid-containing PMN and peripheral opioid analgesia in rats with Freund complete adjuvant-induced hind paw inflammation.

Methods: In circulating PMN of rats treated with G-CSF+SCF and sham-treated rats, opioid peptide content was measured by radioimmunoassay. Expression of adhesion molecules (CD62L, CD49d, CD18), in vitro migration in the Boyden chamber, and infiltrating leukocytes were analyzed by flow cytometry. Chemokine messenger RNA transcription was quantified by LightCycler polymerase chain reaction. Paw pressure threshold was measured at baseline, after cold-water swimming, and after injection of corticotropin-releasing factor.

Results: G-CSF+SCF treatment increased circulating PMN (11-fold, \( P < 0.05 \)). Mobilized PMN had decreased content of \( \beta \)-endorphin but not of Met-enkephalin per cell, down-regulation of CD62L, up-regulation of CD49d (but no change in CD18), and reduced migration toward higher chemokine concentrations (all \( P < 0.05 \)). In the paw, one of four chemokine messenger RNAs was significantly expressed during the first 2 h of inflammation (\( P < 0.05 \)), immigration of PMN and opioid-containing cells was slightly increased (1.5-fold, \( P < 0.05 \)), and baseline paw pressure threshold, as well as paw pressure threshold increases induced by corticotropin-releasing factor and cold-water swimming, were unchanged (\( P > 0.05 \)).

Conclusions: G-CSF+SCF mobilized circulating opioid-containing PMN but had a minor influence on cell migration and peripheral analgesia, probably because of the low expression of chemokines in the inflamed paw and one of the decreased \( \beta \)-endorphin content in PMN.

OPIOIDS are the most powerful drugs for the control of severe postoperative and chronic pain in humans.1 In addition to systemic therapy, analgesia can be produced by local injection of morphine in patients undergoing knee surgery or with chronic arthritis.2 In the synovium, both opioid receptors on sensory neurons and opioid-containing leukocytes are present,3 and these opioid peptides contribute to postoperative analgesia.4 Local opioid-mediated analgesia is devoid of the central side effects of systemic opioid administration (e.g., respiratory depression and nausea).

Studies in models of inflammation have elucidated the underlying mechanisms of peripheral opioid analgesia: polymorphonuclear cells (PMN), monocytes, and lymphocytes are present at the site of an inflammation and contain opioid peptides, such as Met-enkephalin and \( \beta \)-endorphin.5 Opioid-mediated analgesia can be induced in rats by stress (e.g., cold-water swimming [CWS]) or by local injection of corticotropin-releasing factor (CRF),6,7 and it can be antagonized by naloxone or by anti-opioid peptide antibodies.8 Conversely, inhibition of migration of opioid-containing leukocytes significantly impairs peripheral endogenous analgesia.9,10 During the early phase of inflammation, only relatively few opioid-containing leukocytes (mostly PMN) are present. With the duration of inflammation, the number of opioid-containing leukocytes as well as CWS–induced analgesia increases progressively, whereas hyperalgesia at baseline, i.e., pain sensitivity before liberation of opioid peptides by CWS or CRF, is unchanged.11 Based on these findings, we hypothesized that increased migration of opioid-containing PMN into inflamed tissue could increase analgesia, particularly at early stages.

Hematopoietic growth factors, such as granulocyte colony-stimulating factor (G-CSF), mobilize PMN and their precursors from the bone marrow.12 For example, treatment with G-CSF has been shown to enhance recruitment of PMN to the inflamed lung after intratracheal challenge with bacteria or lipopolysaccharide.13,14 Although G-CSF treatment alone is effective, the mobilization of circulating PMN is maximized by using a combined treatment of G-CSF and stem cell factor (SCF).15,16 Recruitment of PMN to inflamed sites is a multistep process involving chemotaxis as well as cell adhesion (fig. 1): chemokines are produced at the site of inflammation and are subsequently presented on the luminal surface of the endothelium.17,18 Circulating PMN recognize most of these chemokines through a single receptor (CXCR2),18 and receptor binding triggers enhanced expression of adhesion molecules.17,19 These molecules reg-
ulate subsequent rolling, sticking, and endothelial transmigr ation of PMN. Consistently, blockage of adhesion molecules inhibits migration of opioid-containing leukocytes to the site of inflammation and significantly decreases CWS- and CRF-induced peripheral analgesia. 3,10

The current study was designed to elucidate mechanisms to enhance the recruitment of opioid-containing PMN to sites of painful inflammation. These PMN have previously been shown to be of major importance in peripheral opioid analgesia. 11 To understand the pathways governing the migration of these PMN to inflamed tissue, we analyzed the expression of adhesion molecules, the migratory capacity of PMN, and the production of chemokines. More specifically, we examined whether PMN mobilization by G-CSF and SCF treatment (1) increases the number of circulating PMN and their opioid peptide content, (2) influences the expression of adhesion molecules (L-selectin, integrins CD18 and CD49d) and the chemotaxis of circulating PMN, and (3) enhances immigration of opioid-containing leukocytes and peripheral opioid analgesia in rats with unilateral hind paw inflammation.

Materials and Methods

Animals and Induction of Inflammation

Animal protocols were approved by the animal care committee of the State of Berlin (Landesamt für Arbeitsschutz, Gesundheitsschutz und Technische Sicherheit, Berlin, Germany) and were in accordance with the guidelines of the International Association for the Study of Pain. 21 Male Wistar rats weighing 140–200 g were injected with 150 μl Freund complete adjuvant (FCA; Calbiochem, La Jolla, CA) into the right hind paw (intraplantar) and developed an inflammation confined to the inoculated paw. Experiments were conducted at 2 h after injection of FCA unless stated otherwise. For all injections, rats were briefly anesthetized with halothane using an open circuit without nitrous oxide.

PMN Mobilization by Hematopoietic Growth Factors

Rats were randomly distributed into cages by the animal caretakers, who did not participate in the experiments and were not informed about the study protocol. Before the distribution of rats, cages were marked with letters (A or B) for either the control or the treatment group. Over 5 consecutive days before FCA inoculation, rats were injected subcutaneously once daily with a combination of 50 μg/kg recombinant rat SCF (Amgen Inc., Thousand Oaks, CA) and 100 μg/kg recombinant human G-CSF (filgrastim; Amgen) in 100 μl glucose 5% (Braun-Melsungen, Melsungen, Germany), as described previously. 15 Rats in the control group were injected subcutaneously with solvent (100 μl glucose 5%).

Fluorescence-activated Cell Staining

Blood Cells. Heparinized blood (20 units/ml, Braun-Melsungen) was obtained by cardiac puncture from deeply anesthetized rats, fixed and lysed as described by the manufacturer (FACS lysis solution; BD Biosciences, Heidelberg, Germany). PMN and mononuclear cells (including monocytes and lymphocytes) were identified by forward–sideward scatter characteristics. For adhesion molecule staining, blood was incubated with fluorescein isothiocyanate–conjugated monoclonal antibodies (mAbs; all at 20 μg/ml and all from BD Biosciences) for 15 min at room temperature in the dark and further processed as described at the beginning of this paragraph. The following antibodies were used: hamster anti-rat CD62L (L-selectin) antibody (isotype: immunoglobulin [Ig] G, clone HRL-1), mouse anti-rat CD49d (VLA-4) mAb (isotype: IgG2a, clone MRα4-1), and mouse anti-rat CD18 mAb (isotype: IgG1, clone WT.3). Controls were incubated with the appropriate isotype-matched antibodies: hamster IgG antibody (clone BB1-3), mouse IgG2a, and mouse IgG1. The intensity of staining was measured by geometric mean fluorescence intensity (MFI). To correct for background nonspecific staining,
Paw Tissue. Paw tissue was dissected from the inflamed paw, leaving the deep flexor tendon in situ. The overlying skin was removed and the tissue was cut into small fragments, enzymatically digested for 60 min, and pressed through a nylon filter to obtain single-cell suspensions as previously described. All samples were stained with Cy-Chrome (BD Biosciences Pharmingen, San Diego, CA) -conjugated mouse anti-rat CD45 mAb (against all hematopoietic cells; 4 µg/ml; BD Biosciences), and mouse anti-rat CD3 phycoerythrin (PMN -endothelin antibody is 0.1% to human β-endorphin and that of the anti-rat β-endorphin antibody is 0% to Met-enkephalin. The sequence of Met-enkephalin is identical in rats and humans. Cytotoxic assay To remove erythrocytes, peripheral blood leukocytes were purified by dextran sedimentation and subsequent hypotonic lysis. The chemokine macrophage inflammatory protein-2 (MIP-2; PeproTech, London, England) was dissolved in complete medium (Roswell Park Memorial Institute 1640 medium; Life Technologies, Paisley, Scotland) containing 100 U/ml penicillin, 10 mg/ml streptomycin, and 10% fetal calf serum (all from Invitrogen, Groningen, The Netherlands) at a dose range of 10−6 to 10−10 m and was placed into the lower well of a Boyden chamber (Costar, Bodenheim, Germany). After trypsin blue staining and counting, 4 × 105 cells in 100 µl complete medium were added to the upper well, separated from the lower well by a cell permeable nylon membrane. After incubation for 60 min at 37°C and 5% CO2, the migrated cell suspension from the lower well was stained with mouse anti-rat CD45 Cy-Chrome (BD Biosciences Pharmingen) Ab (4 µg/ml) and was quantified by TruCOUNT® beads. A migration index was calculated by dividing the number of migrating cells toward MIP-2 by the number of migrating cells under control conditions (medium alone).

Polymerase Chain Reaction mRNA Isolation and cDNA Synthesis. Subcutaneous plantar paw tissue was dissected, and a piece of 25–35 mg from the central inflamed part of the paw was homogenized in Trizol (Invitrogen) as previously described. To synthesize complementary DNA (cDNA), RNA was annealed to 0.125 µM oligodeoxynucleotides reverse transcriptase, and 4 U ribonuclease inhibitor (all from Roche Diagnostics, Mannheim, Germany, except dithiothreitol, Sigma Chemicals) at 42°C for 2 h. Chemokine mRNA Semiquantification by LightCycler PCR. Polymerase chain reaction (PCR) was performed with DNA Sybr green following the manufacturer’s instructions (Roche Diagnostics) using 10 µM of both sense and antisense primers (listed in the next paragraph). cDNA was amplified in a four-step-per-cycle PCR consisting of 95°C for 30 s, primer-specific annealing, elongation, and a fluorescence detection (temperature step [Tm] step (table 1). In pilot experiments, the temperature was determined just below the product-specific melting temperature, and this Tm was incorpo-
rated into the PCR for detection of fluorescence. Chemokine messenger RNA (mRNA) was quantified using duplicate samples and a standard dilution with a known number of copies for a specific PCR product as described previously.Ribosomal protein L19 (RPL-19) was used as a reference gene for quantification.

**PCR Primers.** Primers were intron spanning, and genomic amplification was excluded by agarose visualization. The following primers were used: RPL-19 (sense [SE] 5’ AAT CGC CAA TGC CAA CTC TCG and antisense [as] 5’ TGC TGG TCC ATG AGA ATC CGC TTG); keratinocyte-derived chemokine (SE 5’ AAT GAG CTG CGC TGT CAG TGC and as 5’ TTG GGG ACA CCC TTT AGC ATC); MIP-2 (SE 5’ CAA CCA TCA GGG TAC AGG GGT and as 5’ GTC CGT GAG GGG TCA CCG T); lipopolysaccharide-induced chemokine (SE 5’ CCA AGG TGG AAG TCA TAG CTG GAG GGG TCA CCG T); cytokine-induced chemokine (SE 5’ CAG CCT TCA GGG ACT GTT G and as 5’ AGC TGG ACT TGT CAC TCT TC).

**Measurement of Analgesia**

Nocturnal thresholds were assessed using the paw pressure algometer as previously described (modified Randall-Selitto test; Ugo Basile, Comerio, Italy). Briefly, pressure was applied to the dorsal surface of the hind paw. The average pressure required to illicit paw withdrawal, the paw pressure threshold (PPT), was determined by three consecutive trials both on the inflamed and on the contralateral noninflamed hind paw. PPT determination was performed before treatment (baseline) and 1 min after CWS. Previous dose–response analysis of CRF injection revealed a peak effect at 8 ng that was fully antagonized by intraplantar but not subcutaneous naloxone, demonstrating peripheral opioid-mediated antinociception (data not shown; subcutaneous injections were performed at a remote location on the back to exclude any systemic effect). Data are presented as percent of maximum possible effect (calculated as \[\frac{\text{PPT}_{\text{treated}} - \text{PPT}_{\text{pretreated}}}{250 - \text{PPT}_{\text{pretreated}}} \times 100\%\]; cutoff value of 250 g was used in all experiments to exclude tissue damage). All experiments were performed by an examiner blinded to the treatment protocol.

**Experimental Protocols**

In each experiment, rats were randomly assigned to one of two groups that were injected with G-CSF+SCF (treatment) in 5% glucose or with the solvent only (5% glucose as a control) as described above (Material and Methods, PMN Mobilization by Hematopoietic Growth Factors). On the fifth day and 3 h after the last injection, an examiner blinded to the group assignment injected all rats with FCA intraplantarly and performed the subsequent experiments (removal of paw tissue or behavioral experiments; number of animals per group are given in the figure legends): (1) determination of circulating PMN by flow cytometry and of opioid peptide content by radioimmunoassay; (2) expression of adhesion molecules on circulating PMN by flow cytometry; (3) chemotaxis of circulating PMN in the Boyden chamber; (4) quantification of chemokine mRNA in the inflamed paw by LightCycler (Synthegen, Houston, TX) PCR; (5) quantification of leukocyte subpopulations and of opioid-containing leukocytes in the inflamed paw by flow cytometry; and (6) measurement of analgesia in behavioral experiments before and after CWS or injection of CRF.

**Statistics**

Normally distributed data were analyzed by \(t\) test. If the data lacked equal variance or normality, the Mann-Whitney rank sum test was used. Comparisons of multiple time points were analyzed by analysis of variance (ANOVA) or ANOVA on ranks with a \(t\) post hoc comparison by the Dunnet and Dunn method, respectively. Migration indexes were compared by two-way ANOVA. A \(P\) value less than 0.05 was considered significant. Data are expressed as mean \(\pm\) SEM.

**Results**

**PMN Mobilization and Opioid Peptide Content**

Treatment with G-CSF+SCF significantly increased the number of circulating PMN (fig. 2A; PMN: 11-fold in-
increase; \( P < 0.05 \), Mann–Whitney rank sum test) but not of mononuclear cells (all leukocytes except PMN, i.e., mostly lymphocytes; control: \( 1,487 \pm 182 \) vs. \( 2,548 \pm 510 \) cells/\( \mu l \) blood; \( P > 0.05 \), \( t \) test). For measurement of opioid peptide content, PMN were isolated from circulating blood with a purity of 90 ± 1% (control) and 95 ± 1% (G-CSF + SCF) as determined by flow cytometric staining with the mAb RP-1. Cells were more than 95% viable by trypan blue exclusion. In the PMN fraction, \( \beta \)-endorphin content per cell was significantly decreased and Met-enkephalin content was unchanged in rats treated with G-CSF + SCF (fig. 2B; \( P < 0.001 \), \( P = 0.148 \), respectively, \( t \) test).

**Adhesion Molecule Expression and Migratory Capacity of Mobilized PMN**

In rats treated with G-CSF + SCF, the MFI of CD62L staining was significantly lower, whereas the MFI of CD49d was significantly higher on circulating PMN (figs. 3A and B; \( P < 0.05 \), \( P < 0.001 \), \( t \) test). CD18 expression was unchanged (\( P > 0.05 \), \( t \) test). The *in vitro* cell migration was not influenced by G-CSF + SCF treatment at low MIP-2 concentrations but was significantly lower in rats treated with G-CSF + SCF than in controls at concentrations of \( 10^{-6} \) to \( 10^{-8} \) M (fig. 3C; \( P < 0.05 \), two-way ANOVA).

**Chemokine mRNA Expression in the Inflamed Paw**

mRNA expression of all four chemokines significantly increased with the duration of inflammation (table 2; all \( P < 0.05 \), ANOVA). With the exception of keratinocyte-derived chemokine, this increase did not attain significance during the first 2 h after FCA inoculation.
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Table 2. Chemokine mRNA Expression in the Inflamed Paw

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<tr>
<th>Chemokines</th>
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<tr>
<td></td>
<td>0</td>
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<tr>
<td>KC</td>
<td>&lt; 10</td>
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<tr>
<td>CINC-2</td>
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<td>MIP-2</td>
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Chemokine and reference gene messenger RNA (mRNA) (RPL-19) were quantified by LightCycler polymerase chain reaction from paw tissue at various time points after injection of Freund’s complete adjuvant (FCA) (n = 5 rats per time point). Chemokine mRNA copy numbers were calculated per 10,000 RPL-19 mRNA copies and expressed as mean ± SEM. *P < 0.05, analysis of variance on ranks, each chemokine compared to control (0 h FCA).

CINC-2 = cytokine-induced chemokine 2; KC = keratinocyte-derived chemokine; LIX = lipopolysaccharide-induced chemokine; MIP-2 = macrophage inflammatory protein-2; RPL-19 = ribosomal protein L-19 (housekeeping gene as reference gene).

Fig. 4. Recruitment of polymorphonuclear cells (PMN) to a local site of inflammation. At 2 h after Freund complete adjuvant inoculation, significantly more leukocytes (A, CD45+ cells) and PMN (B, RP-1+ cells, both *P < 0.05) were present in the inflamed paw of rats treated with granulocyte colony-stimulating factor (G-CSF) stem cell factor (SCF) (black bar = solvent; white bar = G-CSF+SCF; *P < 0.05, t test) compared to controls. Monocytes/macrophages (C, ED1+ cells) and lymphocytes (D, CD3+ cells) were unchanged (both P > 0.05 [not significant], all t tests).

Fig. 5. Opioid-containing immune cells and peripheral opioid analgesia in the inflamed paw after mobilization of polymorphonuclear cells (PMN). (A) Flow cytometric analysis showed that opioid-containing leukocytes (3E7+ cells) in the paw were significantly increased by granulocyte colony-stimulating factor (G-CSF) stem cell factor (SCF) treatment (black bar = solvent; white bar = G-CSF+SCF; *P < 0.05, t test; n = 6 or 7). (B) Injection of corticotropin-releasing factor (CRF) elicited similar elevations of paw pressure threshold in control animals (solvent; open symbols) and in animals treated with G-CSF+SCF (filled symbols) in the inflamed paw (square and circle; P > 0.05 [not significant], t test; n = 7). Cold-water swimming stress (CWS; C) produced similar paw pressure threshold increases in treated and untreated animals (P > 0.05 [not significant], t test; n = 7). Neither CRF nor CWS resulted in increases in the paw pressure threshold in the noninflamed, contralateral paws (B and C; inverted triangle = controls; diamond = animals treated with G-CSF+SCF; all P > 0.05 [not significant], t test).

Discussion

In this study, we examined whether endogenous peripheral opioid analgesia in early FCA inflammation can be augmented by systemic immunomodulation. We demonstrated that mobilization of PMN by the hematopoietic growth factors G-CSF and SCF induced a major increase in PMN in the circulation. Mobilized PMN had decreased...
β-endorphin but unchanged Met-enkephalin contents and minor changes in the expression pattern of the adhesion molecules CD62L (L-selectin), CD18 (β₂ integrin), and CD49d (VLA-4). The in vitro migration ability of mobilized PMN was intact in response to low doses but impaired in response to high doses of the chemokine MIP-2. Chemokine mRNA transcription in the paw was hardly detectable in early inflammation. The in vivo migration of opioid-containing leukocytes to the inflamed paw was slightly increased, and endogenous peripheral analgesia was unaltered.

Endogenous opioid-mediated peripheral analgesia has been demonstrated in early stages of an experimental inflammation as well as immediately after surgery in humans. In circulating blood of Wistar rats, lymphocytes constitute the predominant population (60–70% of all leukocytes, 20–30% PMN, 5–10% monocytes). In contrast, PMN are the major leukocyte and opioid-containing population in the inflamed paw during early inflammation (2 and 6 h after intraplantar FCA; of all leukocytes: 60–70% PMN, 20–25% monocytes/macrophages, < 5% lymphocytes). Importantly, because changes in opioid peptide plasma concentrations do not correlate with analgesia, an expansion of opioid-containing leukocytes in the blood will not predict the degree of pain control. In contrast, opioid-containing leukocytes in the inflamed paw induce peripheral opioid analgesia correlating with the number of opioid-containing cells. During early inflammation (2 h after intraplantar FCA), opioid-containing leukocytes are present in lower absolute numbers, and peripheral analgesia is significantly weaker than in late inflammation (96 h after intraplantar FCA), suggesting that analgesia could potentially be augmented by increasing migration of opioid-containing leukocytes to the inflamed paw. Previous studies have shown that recruitment of leukocytes by intraplantar injection of a single stimulatory agent (e.g., leukotriene B₄, formyl-methionyl-leucyl-phenylalanine, or nerve growth factor) induced pain that could be abolished by PMN depletion. On the contrary, in a model of peritonitis (i.e., acetic acid-induced writhing), pain was enhanced in mice depleted of PMN but not of monocytes/macrophages, indicating that PMN conferred analgesia, whereas activation of resident cells (rather than PMN) enhanced inflammatory pain. Furthermore, PMN have been shown to contain opioid peptides such as β-endorphin and Met-enkephalin and to express CRF receptors. Activation of CRF receptors mediates opioid peptide release from leukocytes in vitro and peripheral opioid analgesia in vivo.

In concurrence with previous studies, we have now demonstrated—using flow cytometric quantification—that combined G-CSF+SCF treatment was highly effective in increasing the number of circulating PMN (fig. 2A). Because the 3E7 mAb used for flow cytometry does not permit distinction between β-endorphin and Met-enkephalin, we measured opioid peptide content by radioimmunoassay in purified circulating PMN: cellular β-endorphin content was significantly decreased and Met-enkephalin content was unchanged in mobilized PMN (fig. 2B), which may be explained by observations that G-CSF+SCF treatment induces large numbers of immature PMN and has differential effects on granular protein content. To examine the potential of these cells to migrate to inflamed sites, we first characterized their expression of adhesion molecules. Migration of PMN requires rolling (mediated by selectins) and sticking (mediated by integrins). Blockage of CD62 (selectins) by fucoidin has been shown to reduce migration of opioid-containing leukocytes to the inflamed paw and to essentially abolish analgesia. Although previous studies have demonstrated that mobilization of circulating PMN by G-CSF is highly effective, whether these cells change the expression of adhesion molecules remains controversial. In addition, expression of adhesion molecules has not been studied in combined G-CSF+SCF treatment. This strategy mobilizes an even higher degree of immature PMN than G-CSF treatment alone, which might have a more pronounced effect on the adhesion molecule expression pattern. Similar to studies using G-CSF treatment in humans, we demonstrate here that CD62L expression was only reduced to a minor extent (by 25%; fig. 3B) in rats treated with G-CSF+SCF. This is unlikely to contribute to a reduced capacity to migrate because even in the complete absence of CD62L in genetically deficient mice, PMN migration was reduced by only 50%. The second stage of adhesion involves PMN sticking mediated by CD18 and CD49d expression. In the current study, mobilized PMN had normal CD18 and increased CD49d expression (fig. 3B), as has been shown in human PMN after G-CSF treatment. Taken together, adhesion molecule expression is slightly altered in PMN mobilized by G-CSF+SCF but seems adequate for migration of opioid-containing leukocytes to an inflammatory site. Therefore, enhancement of adhesion molecule expression would not be likely to further augment analgesia.

In addition to adhesion, PMN migration requires intact chemotaxis. A decreased chemotactic capacity of G-CSF-mobilized PMN has been demonstrated in the majority of previous studies. Rodents, recruitment is largely mediated by the chemokine receptor CXCR2 on PMN. Because the four known ligands of the CXCR2 receptor (MIP-2, keratinocyte-derived chemokine, lipopolysaccharide-induced chemokine, and cytokine-induced chemokine 2) all induce PMN recruitment at similar molar concentrations (10⁻⁷ M), we examined in vitro chemotaxis toward one representative CXCR2 ligand (MIP-2) in the current study. We observed a chemotactic defect of mobilized PMN at higher chemokine concentrations (≥ 10⁻⁷ M; fig. 3C).
The question arises whether these concentrations are physiologically relevant in vivo. Chemokines are produced within inflamed tissue, but they exert their chemotactic effects while being bound to glycosaminoglycans on the endothelium in the circulation. This concentration cannot be determined in vivo. In an inflammatory model in mice, chemokine concentrations were determined in an air pouch 2 h after injection of carrageenan, and a concentration of $10^{-8}$ to $10^{-9}$ M was measured. In accordance with this and other studies, we detected only very low levels of chemokine transcription at early stages of inflammation (1 and 2 h vs. 12 h after FCA; table 2) by PCR. Taken together, our results suggest that mobilized PMN show a moderately reduced capacity to migrate toward the chemokine MIP-2 at concentrations potentially relevant in vivo, and only one of four chemokines known to recruit PMN is significantly expressed in early inflammation. These data indicate that augmentation of chemokine expression in the inflamed paw may be a promising strategy to enhance recruitment of opioid-containing PMN and analgesia.

In contrast to our findings in peripheral blood (11-fold increase), PMN mobilization by G-CSF + SCF resulted only in a modest increase (1.4-fold increase) in the number of PMN in the inflamed paw, without significant changes in the number of monocytes/macrophages or of lymphocytes. Previous studies have shown that G-CSF treatment increased the number of circulating PMN (by 3- to 10-fold), but the effect on cell migration to inflamed tissue was variable: G-CSF treatment effectively increased PMN recruitment to the inflamed lung, but PMN recruitment was decreased to human skin or to the cerebrospinal fluid in experimental meningitis. In line with the modest increase of PMN in the inflamed paw by G-CSF + SCF treatment, the number of opioid-containing leukocytes increased to a minor extent (fig. 5A). Consistently, CWS- and CRF-induced analgesia were unchanged by PMN mobilization (figs. 5B and C). Because baseline hyperalgesia was unaltered, the slightly increased number of PMN at the site of inflammation in rats treated with G-CSF + SCF did not intensify pain.

Taken together, the lack of change in analgesia could be attributed to two factors: First, chemokine transcription during the early stage of inflammation is low (table 2), and mobilized PMN have an impaired ability to migrate (fig. 3C). This would explain the rather modest effect of PMN mobilization by G-CSF + SCF treatment in the paw compared to the circulation (figs. 2A and 4B). Second, although the number of opioid-containing (3E7+) cells modestly increased in the paw examined by flow cytometry (fig. 5A), this does not reflect opioid peptide content per cell. In circulating PMN, β-endorphin content per cell was significantly decreased. Because our previous studies in later stages of inflammation indicate that β-endorphin is the major peptide responsible for peripheral opioid analgesia, this may contribute to the net lack of effect on analgesia observed here. In addition, opioid-mediated analgesia in early inflammation might be limited by an insufficient permeability of the perineural barrier to opioid peptides. Finally, low levels of opioid receptor expression or G-protein coupling on the peripheral sensory nerve may account for the unchanged analgesic efficacy.

In summary, our study examined an approach to augment peripheral endogenous analgesia. Treatment with hematopoietic growth factors was effective in expanding the number of circulating PMN, but these cells had a significantly decreased content of β-endorphin. In contrast to circulating blood, G-CSF + SCF increased the number of opioid-containing leukocytes at the site of an inflammation only to a minor extent, and it did not alter peripheral opioid-mediated analgesia. Future strategies to augment the recruitment of opioid-containing PMN and to enhance endogenous analgesia should be directed at increasing chemokine production but not at enhancing the expression of adhesion molecules.

References


