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Monocytes/Macrophages Control Resolution of Transient Inflammatory Pain

Hanneke L. D. M. Willemen,* Niels Eijkelkamp,* Anibal Garza Carbajal,*,§ Huijing Wang,* Matthias Mack,† Jitske Zijlstra,* Cobi J. Heijnen,*,‡ and Annemieke Kavelaars*,‡

**Laboratory of Neuroimmunology and Developmental Origins of Disease, University Medical Center Utrecht, Utrecht, The Netherlands.*
†Department of Internal Medicine II, Regensburg University Hospital, Regensburg, Germany.
‡Department of Symptom Research, University of Texas MD Anderson Cancer Center, Houston, Texas.
§Department for Molecular Human Genetics, Max Planck Institute for Molecular Genetics, Berlin, Germany.

Abstract: **Insights into mechanisms governing resolution of inflammatory pain are of great importance for many chronic pain–associated diseases. Here we investigate the role of macrophages/monocytes and the anti-inflammatory cytokine interleukin-10 (IL-10) in the resolution of transient inflammatory pain. Depletion of mice from peripheral monocytes/macrophages delayed resolution of intraplantar IL-1β- and carrageenan-induced inflammatory hyperalgesia from 1 to 3 days to >1 week. Intrathecal administration of a neutralizing IL-10 antibody also markedly delayed resolution of IL-1β- and carrageenan-induced inflammatory hyperalgesia. Recently, we showed that IL-1β- and carrageenan-induced hyperalgesia is significantly prolonged in LysM-GRK2$^{+/-}$ mice, which have reduced levels of G-protein-coupled receptor kinase 2 (GRK2) in LysM^{+} myeloid cells. Here we show that adoptive transfer of wild-type, but not of GRK2$^{+/-}$, bone marrow-derived monocytes normalizes the resolution of IL-1β-induced hyperalgesia in LysM-GRK2$^{+/-}$ mice. Adoptive transfer of IL-10$^{-/-}$ bone marrow-derived monocytes failed to normalize the duration of IL-1β-induced hyperalgesia in LysM-GRK2$^{+/-}$ mice. Mechanistically, we show that GRK2$^{+/-}$ macrophages produce less IL-10 in vitro. In addition, intrathecal IL-10 administration attenuated IL-1β-induced hyperalgesia in LysM-GRK2$^{+/-}$ mice, whereas it had no effect in wild-type mice. Our data uncover a key role for monocytes/macrophages in promoting resolution of inflammatory hyperalgesia via a mechanism dependent on IL-10 signaling in dorsal root ganglia.**

Perspective: ***We show that IL-10-producing monocytes/macrophages promote resolution of transient inflammatory hyperalgesia. Additionally, we show that reduced monocyte/macrophage GRK2 impairs resolution of hyperalgesia and reduces IL-10 production. We propose that low GRK2 expression and/or impaired IL-10 production by monocytes/macrophages represent peripheral biomarkers for the risk of developing chronic pain after inflammation.***

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According to a recent study by the Institute of Medicine, more than 100 million Americans suffer from chronic pain.[14] One of the limitations for development of novel interventions identified in this report is the limited understanding of the neurobiological pathways leading to transition from acute to chronic pain.

Studies in rodents have revealed that spinal cord microglia, the resident macrophages of the central nervous system, play an important role in the development of chronic pain in models of nerve damage–induced neuropathic pain, diabetic neuropathy, and chronic inflammatory pain.[3,6,27,32,40,45] In addition, it has

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Address reprint requests to Annemieke Kavelaars, PhD, University of Texas MD Anderson Cancer Center, Houston, TX 77030. E-mail: akavelaars@mdanderson.org
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been hypothesized that infiltration of peripheral macrophages into the spinal cord enhances the hyperalgesia in models of chronic pain.[7] A common finding is that proinflammatory cytokines released by activated spinal cord microglia and/or infiltrating macrophages contribute to chronic hyperalgesia, which is a hallmark of these animal models of chronic pain.[5,27,35] Several studies have shown that inhibition of spinal cord proinflammatory cytokine activity or an increase in anti-inflammatory cytokines reduces hyperalgesia in models of chronic pain. In particular, chronic administration of the anti-inflammatory cytokine interleukin-10 (IL-10) has been shown to reduce hyperalgesia in models of neuropathic pain.[16,19,25] However, the role of peripheral monocytes/macrophages and IL-10 in spontaneous resolution of transient inflammatory hyperalgesia has yet to be unraveled.

We recently showed that mice with a cell-specific 50% reduction of G-protein-coupled receptor kinase 2 (GRK2) in lysozyme (Lys)M-positive macrophages/microglia develop markedly prolonged hyperalgesia in response to an intraplantar injection of the cytokine IL-1β, the chemokine CC-chemokine ligand 3 (CCL3), or the inflammatory agent carrageenan. For example, thermal hyperalgesia and mechanical allodynia induced by a single intraplantar injection of the proinflammatory cytokine IL-1β resolves within 1 day in wild-type (WT) mice, but lasts at least 8 days in LysM-GRK2$^{+/-}$ mice.[42,43] Intrathecal (i.t.) administration of the microglial/macrophage inhibitor minocycline reversed this prolongation of hyperalgesia in LysM-GRK2$^{+/-}$ mice, indicating a contribution of spinal cord and/or dorsal root ganglion (DRG) microglia/macrophages in the transition to persistent hyperalgesia.[8,42] The pathophysiological relevance of a reduced level of GRK2 in microglia/macrophages is exemplified by our recent findings that spinal cord microglia/macrophage GRK2 levels are reduced by approximately 40% during chronic inflammatory hyperalgesia and neuropathic pain in WT mice.[8,42] In addition, in patients with the painful chronic inflammatory disease rheumatoid arthritis, the level of GRK2 in circulating mononuclear cells is reduced by 40 to 60%.[18]

Here we investigated the contribution of peripheral monocyte/macrophages in regulating transient inflammatory hyperalgesia using depletion and adoptive transfer strategies on inflammatory hyperalgesia in WT mice. In addition, we investigated the role of peripheral macrophages/monocytes and the anti-inflammatory cytokine IL-10 in the delayed resolution of inflammatory hyperalgesia in LysM-GRK2$^{+/-}$ mice.

Methods

Animals

We used female (aged 10–14 weeks) WT C57BL/6 mice (Harlan, Horst, The Netherlands) or C57BL/6 mice with cell-specific reduction of GRK2 in LysM-positive cells (LysM-GRK2$^{+/-}$).[8,43] For adoptive transfer experiments, WT and GRK2-deficient green fluorescent protein (GFP)-positive bone marrow-derived monocytes (BMDM) were obtained by breeding GRK2$^{+/-}$ mice with CX3CR1$^{gfp/gfp}$ mice (Jackson Laboratories, Bar Harbor, ME). In addition, BMDM from IL-10$^{-/-}$ mice (Jackson Laboratories) were used. Experiments were performed in accordance with international guidelines and approved by the institutional experimental animal committees.

Mice received an intraplantar injection in the hind paw of 5 μL recombinant murine IL-1β (200 ng/mL in saline; PeproTech, Rocky Hill, NJ) or 5 μL λ-carrageenan (1% w/v; Sigma-Aldrich, St. Louis, MO).[8,43] Heat withdrawal latency times were determined using the Hargreaves test (IITC Life Science, Woodland Hills, CA).[10] Mechanical thresholds were determined using the von Frey test with the up-and-down method, as we described.[8,43] All experiments were performed by an experimenter (N.E. or H.W.) blinded to genotype and treatment.

Drug Administration

I.t. injections (5 μL) with goat anti-mouse IL-10 (10 μg in phosphate-buffered saline [PBS]; Sigma-Aldrich), normal goat immunoglobulin G (IgG) (10 μg in PBS; R&D systems, Minneapolis, MN), or human recombinant IL-10 (.5 μg in PBS; Sigma-Aldrich) were performed under light isoflurane anaesthesia as described previously.[8]

Cell Depletion

Mice received intraperitoneal injections with 100 μL anti-CCR2 (MC21; .2 μg/μL[20]) or IgG2b control (BD Biosciences, Franklin Lakes, NJ) at 24 hours and .5 hours before and 10 hours after intraplantar IL-1β or 24 hours and .5 hours before and 24 hours after intraplantar carrageenan. Alternatively, mice received intravenous (i.v.) injections with 200 μL (7 mg/mL) clodronate-liposomes[37] or PBS-liposomes at 24 hours and .5 hours before intraplantar IL-1β.

Adoptive Transfer

BMDM were isolated as described recently.[36] Following Ficoll (GE Healthcare, Pittsburgh, PA) density gradient centrifugation of bone marrow from femora and tibiae, CD115^{+} monocytes were isolated with biotin labeled anti-CD115 antibodies and streptavidin-coupled magnetic beads following the manufacturer's instructions (Miltenyi Biotec, San Diego, CA). IL10$^{-/-}$, WT-CX3CR1$^{gfp/+}$, or GRK2$^{+/-}$-CX3CR1$^{gfp/+}$ BMDM were i.v. injected (3.5 × 10^{6} cells per mouse) or i.t. injected (15,000 cells per mouse). For some experiments, BMDM were labeled with 5 μM carboxyfluorescein succinimidyl ester (Sigma-Aldrich) according to the manufacturer's instructions.

Flow Cytometry

BMDM, blood leukocytes, and cells isolated from the peritoneum were stained with anti-CD115 (eBioscience, San Diego, CA), anti-CCR2 (R&D systems), and anti-CD45. Cells were analyzed on a FACSCanto II flow cytometer using FacsDiva software (BD Biosciences).