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Kruppel-Like Factor 2 Is a Transcriptional Regulator of Chronic and Acute Inflammation

Lalitha Nayak,*† Lediana Goduni,* Yoichi Takami,* Nikunj Sharma,* Parul Kapil,‡ Mukesh K. Jain,* and Ganapati H. Mahabeleshwar*

From the Case Cardiovascular Research Institute, Department of Medicine, Harrington Heart and Vascular Institute, Cleveland; the Division of Hematology and Oncology,† Case Western Reserve University School of Medicine, University Hospitals Case Medical Center, Cleveland; and the Department of Neurosciences,‡ Lerner Research Institute, Cleveland Clinic, Cleveland, Ohio*

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Address correspondence to Ganapati H. Mahabeleshwar, Ph.D., or Mukesh K. Jain, M.D., University Hospitals Harrington Heart and Vascular Institute and Case Cardiovascular Research Institute, Wolstein Research Building, 2103 Cornell Rd., Room 4-537, Cleveland, OH 44106-7290. E-mail: ghm4@case.edu or mukesh.jain2@case.edu.

Although myeloid cell activation is requisite for an optimal innate immune response, this process must be tightly controlled to prevent collateral host tissue damage. Kruppel-like factor 2 (KLF2) is a potent regulator of myeloid cell proinflammatory activation. As an approximately 30% to 50% reduction in KLF2 levels has been observed in human subjects with acute or chronic inflammatory disorders, we studied the biological response to inflammation in $KLF2^{+/-}$ mice. Herein, we show that partial deficiency of KLF2 modulates the *in vivo* response to acute (sepsis) and subacute (skin) inflammatory challenge. Mechanistically, we link the anti-inflammatory effects of KLF2 to the inhibition of NF-κB transcriptional activity. Collectively, the observations provide biologically relevant insights into KLF2-mediated modulation of these inflammatory processes that could potentially be manipulated for therapeutic gain. *(Am J Pathol 2013, 182: 1696—1704; http://dx.doi.org/10.1016/j.ajpath.2013.01.029)*

The process of inflammation is characterized by the recruitment of macrophages to the site of injury.[1] These cells are derived from circulating monocytes and are plastic in nature, with the ability to rapidly modulate their response to the local microenvironment.[2] In the initial phase of the injury response, macrophages typically exhibit a proinflammatory phenotype and release numerous cytokines, chemokines, bactericidal agents, and reactive oxygen and nitrogen intermediates to combat the source of inflammation.[3,4] After removal of the foreign agent, the macrophage also helps in the resolution of inflammation and tissue healing.[5] This process must be carefully controlled, and unbridled inflammation, perhaps due to an overexuberant or protracted response, can lead to tissue injury and other untoward systemic effects.[6] Indeed, there is now compelling evidence that low-grade chronic inflammation can contribute to a broad spectrum of disease states, including dermatitis, arthritis, metabolic disorders, and cardiovascular disease.[7–9]

Activation of the NF-κB pathway is central to the inflammation and resolution phases of the innate immune response.[10] Gram-positive and Gram-negative bacterial cell wall components, such as lipoteichoic acid and lipopolysaccharide (LPS), induce Toll-like receptor signaling that culminates in rapid activation of NF-κB followed by expression of downstream target genes that initiate the initial inflammatory response.[11–13] Subsequently, NF-κB induces pathways that, in a negative feedback manner, limit its activity and facilitate the resolution of inflammation.[14,15] Failure to limit NF-κB activation leads to chronic inflammation, as has been observed in inflammatory bowel disease, metabolic syndrome, atherosclerosis, rheumatoid arthritis, and psoriasis.[16–19] The importance of this pathway is underscored by experimental and clinical observations demonstrating that inhibition of the NF-κB pathway (eg, through the use of anticytokine therapies) can ameliorate disease.[20–23]

Kruppel-like factors (KLFs) are a subclass of the zinc-finger family of DNA-binding transcription factors that regulate diverse biological processes.[24] KLF2 was initially

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identified by the Lingrel laboratory and was named for its high expression in the lung.[25] Subsequent work from our group and others has implicated this factor as a negative regulator of inflammation and NF-κB activity.[26–28] Studies in human subjects with inflammatory conditions, such as sepsis, reveal that cellular levels of KLF2 are reduced by approximately 30% to 50%.[27] To recapitulate this experimentally, we undertook studies in KLF2 hemizygous mice. Our findings show that partial deficiency of KLF2 alters the biological response in diverse models of acute inflammation. These findings, coupled with previous work in the context of chronic inflammatory disease states, such as atherosclerosis,[29] speak strongly to the importance of KLF2 in the biological response to inflammation.

Materials and Methods

Materials

LPS, carrageenan, and phorbol ester 12-*O*-tetradecanoylphorbol-13-acetate (TPA) were purchased from Sigma-Aldrich (St. Louis, MO). Recombinant mouse interferon γ (IFN-γ) protein and tumor necrosis factor α (TNF-α)/monocyte chemoattractant protein (MCP)-1 enzyme-linked immunosorbent assay (ELISA) kits were obtained from R&D Systems (Minneapolis, MN). A myeloperoxidase colorimetric activity assay kit was obtained from BioVision (Milpitas, CA). Thioglycolate broth and anti-MAC3 antibody were obtained from BD Pharmingen (Franklin Lakes, NJ). Anti-B220/CD45R antibody was purchased from BD Pharmingen and anti-CD3 antibody from Abcam Inc. (Cambridge, MA). Rabbit polyclonal anti-p300 and anti-PCAF antibodies were obtained from Santa Cruz Biotechnology (Santa Cruz, CA). The RAW264.7 cell line was purchased from ATCC (Manassas, VA). A luciferase reporter assay system was obtained from Promega (Madison, WI). All other chemicals and reagents used were of analytical grade and were obtained from commercial sources.

Cell Culture

Primary mouse macrophages were obtained from the peritoneal cavity by inducing peritonitis with 3% thioglycolate broth in 8- to 12-week-old mice as described earlier.[27] These primary peritoneal macrophages were maintained in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum, 100 U/mL of penicillin, 100 μg/mL of streptomycin, and 2 mm of glutamine in a humidified atmosphere of 5% CO_2 and 95% air at 37°C. The RAW264.7 cell line was used for *in vitro* experiments and was maintained under aseptic conditions.

Experimental Mouse Models

The KLF2 hemizygous ($KLF2^{+/-}$) mouse line used in this study was generated as described previously.[30] Mouse colonies used in this study were maintained in a clean animal facility, and all animal experimentation was approved by the Case Western Reserve University Institutional Animal Care and Use Committee (Cleveland, OH).

TPA-Induced Cutaneous Inflammation Model

TPA-induced cutaneous inflammation experiments were performed as described previously.[31] Briefly, ear fur from wild-type (WT) and KLF2 hemizygous mice was removed 2 days before the experiment. The right ear was treated twice at an interval of 24 hours with TPA (2.5 μg of TPA in 20 μL of acetone). The left ear was treated similarly with acetone alone and served as a control. The mice were sacrificed 24 hours after the second TPA application. Ear weight was recorded using an analytical balance. The ear tissues were either frozen to analyze myeloperoxidase activity or fixed with 4% paraformaldehyde to perform immunohistochemical analysis using anti-MAC3 antibody to detect macrophage infiltration. Lymphoctye infiltration was also assessed immunohistochemically using B-cell (anti-B220/CD45R antibody) and T-cell (anti-CD3 antibody) markers.

Carrageenan-Induced Inflammation Model

A carrageenan-induced paw edema and inflammation experiment was performed as described elsewhere.[26] Briefly, the left hindfoot pads of WT and KLF2 hemizygous mice were injected with 0.1 mL of 1% carrageenan (λ-carrageenan, type IV). The right hindfoot pad received 0.1 mL of saline and was used as control. After 24 hours of injection, mice were euthanized and paw weight was recorded using an analytical balance, and images were obtained. These paws were fixed in 4% paraformaldehyde, and immunohistochemical analyses were performed using anti-MAC3 antibody to detect macrophage infiltration.

Thioglycolate-Induced Inflammation Model

WT and KLF2 hemizygous mice were subjected to thioglycolate-induced peritonitis as described previously.[27] Briefly, WT and KLF2 hemizygous mice were injected intraperitoneally with 1 mL of sterile 3% thioglycolate broth per mouse. Mice were euthanized 72 hours after thioglycolate injection, and the peritoneal cavity was flushed with 5 mL of sterile PBS and 1 mL of air to harvest cells. The total number of macrophages that responded to the site of inflammation was counted as an indicator of inflammatory status and was used for further biochemical analysis.

LPS-Induced Sepsis Model

WT and KLF2 hemizygous mice were subjected to the LPS-induced sepsis model as described previously.[27] Briefly, WT and KLF2 hemizygous mice were injected i.p. with 23 mg/kg of LPS. These mice were monitored for rectal temperature, blood pressure, and heart rate after LPS injection. Plasma TNF-α and MCP-1 levels were quantified using an ELISA kit. The shock index was determined using the following formula: Shock Index = Heart Rate/Systolic Blood Pressure.

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