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Sulindac, a nonsteroidal anti-inflammatory drug, selectively inhibits interferon-γ-induced expression of the chemokine CXCL9 gene in mouse macrophages

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Abstract

Sulindac, a non-steroidal anti-inflammatory drug, has been shown to exert an anti-tumor effect on several types of cancer. To determine the effect of sulindac on intracellular signaling pathways in host immune cells such as macrophages, we investigated the effect of the drug on interferon gamma (IFNγ)-induced expression of signal transducer and activator of transcription 1 (STAT1) and other genes in mouse macrophage-like cell line RAW264.7 cells. Sulindac, but not aspirin or sodium salicylate, inhibited IFNγ-induced expression of the CXC ligand 9 (CXCL9) mRNA, a chemokine for activated T cells, whereas the interferon-induced expression of CXCL10 or IFN regulatory factor-1 was not affected by sulindac. Luciferase reporter assay demonstrated that sulindac inhibited IFNγ-induced promoter activity of the CXCL9 gene. Surprisingly, sulindac had no inhibitory effect on IFNγ-induced STAT1 activation; however, constitutive nuclear factor κB activity was suppressed by the drug. These results indicate that sulindac selectively inhibited IFNγ-inducible gene expression without inhibiting STAT1 activation.

Keywords: Sulindac; NSAID; Interferon-γ; Chemokine; CXCL9; Macrophages; Gene expression; STAT1; NF-κB

Mononuclear phagocytes play an essential role in the regulation of host defense and homeostasis, and in the development of chronic inflammatory diseases. Interferon gamma (IFNγ), a cytokine produced by NK cells and type I helper T cells, is a potent macrophage-activating stimulus that induces the expression of many genes necessary for the execution of host defense function including MHC class II molecules, nitric oxide synthase 2, and chemokine CXC ligand 9 (CXCL9) and CXCL10 [1,2]. The IFNγ-inducible chemokines CXCL9 and CXCL10, known as monokine induced by gamma IFN (Mig) and IFN-inducible protein 10 kDa, respectively, are chemoattractants for activated T cells [3,4]. The expression of these chemokines has been observed in a wide variety of chronic inflammatory disorders such as multiple sclerosis, rheumatoid arthritis, and allograft rejection [5]. These chemokines have been also shown to exhibit anti-tumor activity in rodent model systems [6].

Sulindac, a nonsteroidal anti-inflammatory drug (NSAID), has been shown to reduce the incidence of colorectal adenomas and carcinomas, and is used as a cancer chemopreventive agent for those disorders (see review [7]). Although sulindac inhibits both cyclooxygenase-1 (COX-1), and COX-2 and thereby inhibits prostaglandin synthesis, the pharmacological action of sulindac on the anti-tumor activity has been attributed to inhibition of cell growth and induction of apoptosis in tumor cells [8].

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Previous studies have demonstrated that sulindac inhibits NF-κB activation by inhibiting IκB kinase [9] and down-regulates the phosphorylation of extracellular signal-regulated kinase (ERK)1/2 by inhibiting its upstream kinase, MEK1/2 [10]. These lines of evidence suggest that sulindac modulates signaling pathways that are essential for many cellular functions including cell growth, survival, and gene expression. Although the pharmacological action of sulindac on tumor cells has been extensively explored, the biochemical mechanisms underlying the effect of sulindac on signaling pathways in host immune cells such as macrophages are poorly understood.

The present study was undertaken to evaluate the effect of sulindac on IFNγ-induced gene expression in mouse macrophages. The results demonstrate that sulindac selectively inhibited IFNγ-induced expression of CXCL9 gene in mouse macrophage-like RAW264.7 cells without affecting IFNγ-induced STAT1 activation. This selective inhibition appears to have been mediated by down-regulation of constitutive NF-κB activity, which cooperates with IFNγ-induced STAT1-dependent transcription. Our results suggest that, although sulindac exhibits a direct apoptotic activity against tumor cells, the NSAIDS may also affect host immune responses by down-regulating IFNγ-inducible gene expression.

Materials and methods

Reagents. Sulindac, aspirin, and sodium salicylate were obtained from Sigma–Aldrich Co. (St. Louis, MO). Recombinant mouse IFNγ was purchased from BioSource International Inc. (Camarillo, CA). Rabbit polyclonal antibodies against mouse STAT1α (M-23), NFkB1(NLS), and RelA (A) were obtained from Santa Cruz Biotechnology (Hercules, CA). FuGene transfection reagent was purchased from Roche (Nutley, NJ).

Cell culture. Cells of the mouse macrophage-like cell line RAW264.7 were cultured in Dulbecco's modified Eagle's medium (DMEM) containing L-glutamine, penicillin–streptomycin, and 10% FBS, and subcultured every three days.

Preparation of RNA and Northern hybridization analysis. Total cellular RNA was extracted by the guanidine isothiocyanate–cesium chloride method [11]. Samples of total RNA (5 μg) were separated on a 1% agarose–2.2 M formaldehyde gel and subsequently blotted onto a MAGNA nylon membrane. The blots were prehybridized at 42 °C in 50% formamide, 1% SDS, 5× SSC, 1× Denhardt's solution, 0.25 mg/ml denatured salmon sperm DNA, and 50 mM sodium phosphate (pH 6.5), and then hybridized with a radiolabeled cDNA plasmid probe. The cDNA probes used for mouse CXCL9/Mig and CXCL10/IP-10, interferon regulatory factor-1 (IRF-1), and rat glyceraldehyde-3-phosphate dehydrogenase (GAPDH) were described previously [12].

Luciferase reporter assay. The luciferase reporter construct Mig-328, in which a 0.3-kb portion of the 5′-flanking sequence of the mouse CXCL9/Mig gene [13] was cloned into the pGL2 luciferase reporter plasmid (Promega, Madison, WI), was described previously [14]. The pGL3 control luciferase plasmid was also obtained from Promega. Cells were transiently transfected with luciferase reporter plasmids and pRL-TK reference Renilla luciferase plasmid (Promega) by using FuGene transfection reagents (Roche, Nutley, NJ) according to the manufacturer's instructions.

Electrophoretic mobility shift assay (EMSA). Nuclear extracts were prepared as described previously [15] by using a modification of the method of Dignam et al. [16]. The following oligonucleotides were used for the EMSA (sense strand):

MigγRE [13,17], 5′-gatcCCTTACTATAAACTCCCCGTTTATG TGAAATGGA-3′; Mig κB2 [17], 5′-tcgaAGTAGGGTTTTCCC CAGGA-3′; IRF-1 GAS [18] , 5′-tcgaGCCTGATTTCCCCGAAAT GAGGC-3′. Binding reactions and antibody supershift assay were described previously [15,19]. The dried gels were analyzed by autoradiography and by phosphorescence detection for quantitative analysis of DNA-binding activity.

Results

Nonsteroidal anti-inflammatory drug sulindac selectively inhibited IFNγ-induced expression of CXCL9 mRNA in mouse macrophage-like cell line RAW264.7

To examine whether sulindac could inhibit IFNγ-induced expression of chemokine CXCL9, we pretreated mouse macrophage-like RAW264.7 cells with various concentrations of sulindac for 30 min and then stimulated them with IFNγ for 4 h. After the stimulation, total RNA was prepared and analyzed by Northern hybridization using a cDNA probe detecting CXCL9 mRNA (Fig. 1A). Stimulation of RAW cells with IFNγ-induced CXCL9 mRNA expression (lane 2), and the level of CXCL9 mRNA expression was significantly suppressed

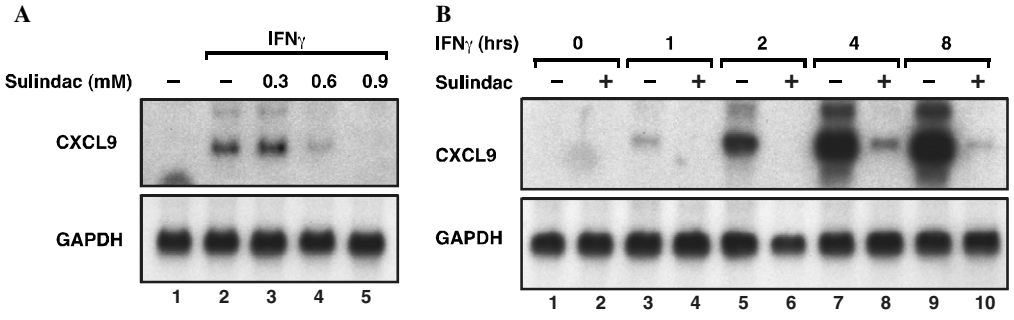

Fig. 1. The nonsteroidal anti-inflammatory drug sulindac inhibits IFNγ-induced expression of CXCL9 mRNA in RAW264.7 cells. (A) Mouse macrophage-like cells RAW264.7 were pretreated with various concentrations of sulindac as indicated for 30 min and then treated or not with IFNγ (10 ng/ml) for 4 h before preparation of total RNA. Specific mRNA levels were analyzed by Northern hybridization. Five microgram of total RNA was analyzed in each lane. (B) RAW264.7 cells were either pretreated or not with sulindac (0.9 mM) for 30 min and then stimulated with IFNγ (10 ng/ml) for various periods of time as indicated. The cultures without IFNγ treatment are shown as 0 h in the figure. Specific mRNA levels were analyzed by Northern hybridization as indicated above. Similar results were obtained from 3 independent experiments.