Supplemental Figure legends

Supplemental Figure S1.

Figure S1. SU6656, the Src tyrosine kinase inhibitor, down-regulates IL-13-stimulated 15-LO gene expression in monocytes

Monocytes ($5x10^6$/group) were either directly treated with IL-13 for 24 h or pretreated with the Src kinase inhibitor SU6656 (A and B) for 30 min at various indicated doses followed by stimulation with 1 nM IL-13 for 24 h. In A, total cellular RNA extracts were prepared and subjected to quantitative real-time PCR analysis. After normalization with GAPDH amplification, the fold induction of 15-LO mRNA expression for different groups was plotted. Data were collected from two similar experiments and are shown as means ± data range. In B, 50 μg of the postnuclear lysates were resolved by 8% SDS-PAGE and subjected to immunoblotting with a 15-LO specific antibody. The 15-LO blot was stripped and reprobed with β-tubulin antibody (lower panel of B) to assess equal loading. The results in B are representative of three independent experiments.

Supplemental Figure S2.

Figure S2. Src tyrosine kinase activity is required for IL-13-dependent p38MAPK activation but not for PKCδ activation

Human monocytes ($10x10^6$/group) (A and B) were pretreated with Src kinase inhibitors SU6656 or PP2 or its inactive structural analog PP3 for 30 min at various indicated doses followed by IL-13 treatment for 15 min. Postnuclear extracts were prepared and subjected to IP with p38MAPK (A) and PKCδ (B) antibodies respectively. The immunoprecipitates were resolved by SDS-PAGE for immunoblotting with the anti-

phosphotyrosine antibody, PY99 (upper panels of A and B). The lower panels show the expression of p38MAPK (A) and PKCδ (B) protein levels of the cytosolic immunoprecipitates.

Supplemental Figure S3.

Figure S3. Src PTK isoforms Fgr and Lyn are not phosphorylated/activated by IL-13 stimulation in human monocytes

Monocytes ($5x10^6$/group) (A) and ($10x10^6$/group) (B and C) were either incubated in medium alone or directly treated with IL-13 (2 nM) for 15 min. Panel A shows the constitutive expression of the Src PTKs in lysates of human monocytes. In B and C, postnuclear extracts were prepared and subjected to IP with Fgr (rabbit polyclonal) (B) and Lyn (rabbit polyclonal) (C) antibodies respectively. The immunoprecipitates were resolved by SDS-PAGE for immunoblotting with the anti-phosphotyrosine antibody, PY99 (mouse monoclonal). HRP-conjugated donkey anti-mouse, preabsorbed secondary antibody from Affinity Bioreagents Inc. (Golden, CO) was used to develop the blots in the upper panels. The blots were subsequently stripped and reprobed with Fgr and Lyn antibodies respectively to confirm equal immunoprecipitation (lower panels of B and C).

Supplemental Figure S4.

Figure S4. Hck controls 15-LO gene expression in IL-4-stimulated monocytes

Monocytes ($5x10^6$/group) were pre-treated with Hck-specific antisense (AS) or sense (S) ODNs at 10 μM for 48 h prior to stimulation with IL-4 (670 pM) for 24 h. Total cellular RNA extracts were prepared and subjected to quantitative real-time PCR analysis. After normalization with GAPDH, the fold induction of 15-LO mRNA for different groups was

plotted. Data are the means ± S.D. (n=3). Significant differences were determined by comparing the Hck antisense (AS) or sense (S) ODN treated groups to the IL-4-stimulated control (*p<0.008).

Supplemental Figure S5.

Figure S5. Proposed model for IL-13-mediated regulation of 15-LO gene expression in primary human monocytes

Several signal transduction pathways downstream of the IL-13 receptor regulate the 15-LO gene expression. These include: (1) Jak2 and Tyk2 activation (tyrosine phosphorylation) after IL-13 induction (Roy and Cathcart, 1998); (2) Phosphorylation of receptor components (IL-13Rα1 and IL-4Rα), association of Jaks (Jak2, Tyk2) with the receptor components and the tyrosine phosphorylation of Stats (Stat1 and Stat3 tyrosine phosphorylation is shown here) (Roy et al., 2002); (3) The IL-13Rα1-Tyk2-mediated signaling pathway that is required for ERK1/2 MAPK activation and the ERK1/2 MAPK-dependent transcriptional regulation of 15-LO via CREB and Egr-1 transcription factors (Bhattacharjee et al., 2010); (4) p38MAPK-mediated serine phosphorylation of Stat1 and Stat3 and Stat-dependent transcriptional regulation of 15-LO (Xu et al., 2003); (5) Formation of a cytosolic signaling complex containing PKCδ, tyrosine phosphorylated Stat3 and p38MAPK that is required for Stat3 serine phosphorylation, a critical regulatory step in 15-LO gene transcription (Bhattacharjee et al., 2006); A. Bhattacharjee and M.K. Cathcart, unpublished}; and (6) IL-4Rα-Jak2-mediated signaling pathway for the phosphorylation/activation of the Src kinase Hck in alternatively activated monocytes/macrophages that leads to activation of the Hck-MKK3/6-p38MAPK signaling cascade followed by the Stat1 and Stat3 serine phosphorylation and 15-LO gene

transcription. This is the focus of this manuscript and shown here by the dashed-line outline.

References

Bhattacharjee, A., Mulya, A., Pal, S., Roy, B., Feldman, G. M. and Cathcart, M. K. (2010). Monocyte 15-lipoxygenase gene expression requires ERK1/2 MAPK activity. *Journal of immunology* **185**, 5211-24.

Bhattacharjee, A., Xu, B., Frank, D. A., Feldman, G. M. and Cathcart, M. K. (2006). Monocyte 15-lipoxygenase expression is regulated by a novel cytosolic signaling complex with protein kinase C delta and tyrosine-phosphorylated Stat3. *J Immunol* **177**, 3771-81.

Roy, B., Bhattacharjee, A., Xu, B., Ford, D., Maizel, A. L. and Cathcart, M. K. (2002). IL-13 signal transduction in human monocytes: phosphorylation of receptor components, association with Jaks, and phosphorylation/activation of Stats. *J Leukoc Biol* **72**, 580-9.

Roy, B. and Cathcart, M. K. (1998). Induction of 15-lipoxygenase expression by IL-13 requires tyrosine phosphorylation of Jak2 and Tyk2 in human monocytes. *J Biol Chem* **273**, 32023-9.

Xu, B., Bhattacharjee, A., Roy, B., Xu, H. M., Anthony, D., Frank, D. A., Feldman, G. M. and Cathcart, M. K. (2003). Interleukin-13 induction of 15-lipoxygenase gene expression requires p38 mitogen-activated protein kinase-mediated serine 727 phosphorylation of Stat1 and Stat3. *Mol Cell Biol* **23**, 3918-28.