**Induction of 15-Lipoxygenase Expression by IL-13 Requires Tyrosine Phosphorylation of Jak2 and Tyk2 in Human Monocytes**

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The enzyme 15-lipoxygenase (15-LO) participates in the dioxygenation of polyenoic fatty acids. This activity leads to the degradation of mitochondrial membranes during reticulocyte differentiation, the production of pro- and anti-inflammatory mediators by a variety of cell types, and the oxidation of lipids in atherosclerotic lesions. The cytokines, IL-4 and IL-13, are reported to induce the expression of 15-LO in human peripheral blood monocytes. In this report we explore the signaling mechanisms involved in the IL-13-mediated induction of 15-LO expression. First we demonstrate that the delayed induction of 15-LO requires continuous stimulation of monocytes for a minimum period of 12 h. We also found that tyrosine kinase inhibitors blocked the induction of 15-LO in a dose-dependent manner. By immunoprecipitation and antiphosphotyrosine blotting experiments, IL-13 was shown to induce tyrosine phosphorylation of Jak2 and Tyk2, but not Jak1 or Jak3, within 5 min of treatment in human monocytes. To investigate whether the early induction of tyrosine phosphorylation of both Jak2 and Tyk2 was ultimately involved in 15-LO expression, we generated antisense oligodeoxyribonucleotides (ODNs) against Tyk2 and Jak2. We employed a cationic lipid-mediated delivery technique to transfect the monocytes and found that both antisense ODNs inhibited expression of their target proteins by 75–85%. The treatments were specific and did not affect the expression of each other. Furthermore, the antisense ODNs to Jak2 and Tyk2 both inhibited the induction of expression of 15-LO in monocytes treated with IL-13. Parallel experiments with sense ODNs to Jak2 and Tyk2 did not affect their protein levels or the induction of 15-LO by IL-13, and down-regulation of Jak1 also did not affect expression of 15-LO. Our results suggest the novel finding that IL-13 can induce tyrosine phosphorylation of both Jak2 and Tyk2 in primary human monocytes. This occurs as an early and essential signal transduction event for the IL-13-mediated induction of 15-LO expression. These data represent the first characterization of upstream kinases involved in the induced expression of 15-LO.

Among the members of the lipoygenase family (EC 1.13.11.12), 15-lipoxygenase and the closely related leucocyte type 12 lipoygenases are of special interest because of their ability to oxidize unsaturated fatty acids (1, 2) and also more complex substrates such as phospholipids, cholesterol esters (2–4), and lipoproteins (5, 6). In human leucocytes, catalysis of arachidonic acid oxidation by 15-LO leads to the formation of 15(S)-hydroperoxyeicosatetraenoic acid, 15(S)-hydroxyeicosatetraenoic acid, and lipoxins A4 and B4 (7–10). These compounds possess a wide range of biological activities. Both 15(S)-hydroxyeicosatetraenoic acid and lipoxins A4 are potent endogenous anti-inflammatory molecules in view of their capacities to suppress white cell chemotaxis, adherence, activation, and to specifically antagonist the functional responses of pro-inflammatory 5-LO derivatives, the leukotrienes (11–13).

Products of 15-LO-mediated oxidation of linoleic acid have been identified in human and rabbit atherosclerotic lesions and likely result from the action of 15-LO on lipoprotein lipids (6, 14). It has been reported that 15-LO is expressed at a high level in lipid-loaded macrophages of atherosclerotic lesions (14–16) and to a lesser extent in subtypes of arterial smooth muscle cells (17); however, the role of this enzyme in the pathogenesis of cardiovascular disease is far from clear. Because circulating blood monocytes of normal individuals do not express 15-LO (18), the enzyme must be induced during activation or macrophage differentiation in the tissue.

Numerous lymphobones are known to modulate the inflammatory response through their actions on monocytes (19, 20). The involvement of the local environment of the monocyte in influencing 15-LO expression is supported by studies showing that IL-4 and IL-13 are specific inducers of the enzyme (18, 21). Attempts to study the molecular signaling mechanisms using monocytic cell lines have not been successful, as the cell lines failed to show similar induction of 15-LO when stimulated with interleukins (22). Of several permanent human hematopoietic and epithelial cell lines, only the lung epithelial cell line, A549, showed induction of 15-LO, even though all the cell lines tested were positive for the presence of cell surface receptors for both IL-4 and IL-13 (22).

Several reports support the view that interleukins exert their effects through the mediation of different combinations of receptor subunits in different cell lines and thus activate different combinations of Jak and signal transducers and activators of transcription (STATs) (23–26). Although IL-4Ra (140 kDa) was shown to be a substrate for both IL-4- and IL-13-dependent tyrosine phosphorylation, the other constituents of the receptor complex in different cells and cell lines are not yet clear (27). Two different IL-13 receptors are reported, one with lower affinity toward IL-13 (Kd = 3–10 nM) having a molecular mass of 56–68 kDa (28), termed IL-13Ra, and the other with lower affinity toward IL-13 (Kd = 3–10 nM) having a molecular mass of 56–68 kDa (28), termed IL-13Ra, and the other with

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1 The abbreviations used are: LO, lipoxygenase; ODN, oligodeoxyribonucleotide; STAT, signal transducers and activators of transcription; PAGE, polyacrylamide gel electrophoresis; BCS, bovine calf serum; DMEM, Dulbecco’s modified Eagle’s medium; DDAB, dimethyl dioctadecyl ammonium bromide; DOPE, dioleoylphosphatidylethanolamine; IL, interleukin; PVD, polyvinylidene difluoride; HPLC, high performance liquid chromatography.
our attempts to inhibit the activity and expression of classical transduction in some cells, other IL-4-responsive cell lines (IL-2Rα  
(38)). Although IL-2Rα is required for IL-4-mediated signal transduction in some cells, other IL-4-responsive cell lines (e.g. 
plasmacytoma B9 and renal cell lines) exist that do not express IL-2Rα (39). Expression of IL-2Rα on human monocytes has 
been shown to be very low (40) or absent (24), thus leading to the suggestion that the functional composition of IL-4 and 
IL-13 receptor complexes may vary from cell type to cell type and that IL-2Rα is not absolutely necessary for receptor signaling.

Different groups have utilized antisense oligodeoxyribonucleotides (ODNs) to inhibit the endogenous level of expression 
of different enzymes, including the Jaks and STATs, in various cells and cell lines (41–43). Jak2 antisense ODN treatment 
resulted in a reduction of expression of up to 46% in human and murine cell lines and normal human progenitor 
cells (41). The level of expression of STAT1α was reduced in the astrogliaoma cell line (CH225-MG) as well as in gliomerular 
mesangial cells using antisense ODN specific to STAT1α (42). Our attempts to inhibit the activity and expression of classical 
protein kinase C (44) and cytosolic phospholipase A2 (45) using antisense ODNs met with an even higher level of inhibition (up to 
80%) as this approach worked more efficiently in nonproliferating, rapidly pinocytosing monocytes.

In the current study, we examined the signaling intermediates triggered by IL-13 in human monocytes and assessed their 
involvement in the induction of expression of 15-LO. Our data indicate that the induction of 15-LO was delayed and depend-
ent on tyrosine kinases. Using immunoprecipitation studies, we demonstrated that IL-13 induced tyrosine phosphorylation 
of Jak2 and Tyk2. The induction of expression of 15-LO could be inhibited by using antisense ODNs against Jak2 and Tyk2, 
suggesting their involvement in the IL-13-signaling pathway in human monocytes.

**EXPERIMENTAL PROCEDURES**

**Chemicals**

Genistein, daidzein, tyrophostin 23, and tyrophostin 1 were purchased from Biomol Research Laboratories (Plymouth, PA). All 
the drugs were dissolved in Me2SO. All the reagents were made as 1000-fold concentrated stock solutions and stored at −20 °C before use.

Recombinant human IL-13 was purchased from Upstate Biotechnology, Lake Placid, NY. Antibody against rabbit reticulocyte 15-LO cross-
reacting with human 15-LO was raised in sheep and was a kind gift of Dr. Joseph Cornelici, Parke-Davis.

Rabbit antisera against Jak1, Jak2, Tyk2, and Jak3 were purchased from either Upstate Biotechnology or Santa Cruz Biotechnology, Inc., CA. Each one of the Jak antibodies was essentially noncross-reactive with the other members of the Jak family and recognized corresponding 
antigens under native (in 1% Triton X-100 extracts) as well as dena-

**Detection of tyrosine-phosphorylated proteins by immunoblotting, a mixture (1:1) of anti-phosphotyrosine antibodies PY-20 (Santa 
Cruz Biotechnology, Inc., CA) and 4G-10 (UBI, Lake Placid, NY) were used, both at dilutions of 1:1000. Antibody to phosphotyrosine, PY-99 (Santa Cruz Biotechnology, Inc.), was used to immunoprecipitate ty-
rosine-phosphorylated proteins.

**Methods**

**Isolation of Human Monocytes**—Human blood monocytes were iso-
lated from heparinized whole blood by sequential centrifugation over a 
Ficoll-Paque solution and adherence to serum-coated tissue culture 
flasks as described previously (46). Nonadherent cells were removed by 
gentle washing, and adherent cells were collected after releasing with 5 
mM EDTA and plated in tissue culture plates (Costar, Cambridge, MA) 

methods of DMEM-BCS medium and incubated for up to 36 h. Subse-
sequently, the cells were washed three times with PBS to remove the 
traces of DMEM, 10% BCS. The plates were placed on ice, and the cells 
were lysed using 200 µl of lysis buffer (1% Triton X-100, 150 mM NaCl, 
50 mM Tris-HCl, pH 7.4, 1 mM phenylmethylsulfonyl fluoride and 10 µl 
of protease inhibitor mixture (Sigma/1 ml of lysis buffer). After 30 min, 
the lysates were centrifuged at 9300 × g. The supernatant was collected, 
and the protein concentration was determined using the Bio-
Rad protein assay kit and loaded on a 7.5% SDS-PAGE gel (50 µg of 
ylate/well). The proteins were transferred to a PVDF membrane (0.2 
(μm) (Bio-Rad) using a Trans-Blot SD electrophoretic transfer cell (Bio-
Rad). The membrane was blocked in 3% nonfat milk in 20 mM Tris-
HCl, pH 7.4, 150 mM NaCl, and 0.1% Tween 20 for 1 h at room temperature 
and was probed with antibody to rabbit reticulocyte 15-LO (diluted 
1:2000 in 20 mM Tris-HCl, pH 7.4, 150 mM NaCl, and 0.1% Tween 20 for 1 
h at room temperature. This antibody was shown to be specific for 
15-LO and does not cross-react with 5-LO (47). A horseradish peroxi-
dase-labeled secondary antibody (ICN Biocemicals, Cleveland, OH) 
diluted 1:5000 in 20 mM Tris-HCl, pH 7.4, 150 mM NaCl and 0.1% 
Tween 20 was added for 1 h. The hybridization signal was detected 
using Enhanced Chemiluminescence (ECL) detection reagents (Pierce) 
according to the manufacturer’s guide and followed by autoradiography.

**Immunoprecipitation and Western Blotting**—Freshly isolated monocytes were pretreated with activated sodium vanadate solution (5 mM 
final concentration) for 30 min followed by treatment with IL-13 (250 
(μM) for 5 min. The treated cells were immediately lysed at 50 × 10^6/ml 
in lysis buffer (1% Triton X-100, 150 mM NaCl, 50 mM Tris, pH 7.4, 5 mM sodium pyrophosphate, 1 mM sodium orthovanadate, 
500 µM phenylmethylsulfonyl fluoride, and 1:100 diluted protease inhibitor 
mixture). After 30 min on ice, the extracts were centrifuged at 9300 × 
g for 15 min at 4 °C, and the post-nuclear extract (500 µg/ml) was 
incubated with the relevant antibody for 2 h at 4 °C with constant 
rotation. Immune complexes were collected using Sepharose-protein A 
beads (20 µl packed volume). After washing 5 times (10 min each) with 
lysis buffer, the immune complexes were released in SDS sample buffer 
and analyzed by SDS-PAGE followed by electrophoretic transfer to 
PVDF membranes (Bio-Rad). The membranes were then probed with either antibody to phosphotyrosine or individual antibodies to Jak/Tyk 
kinasas and developed using ECL. For immunoprecipitation experi-
ments using anti-phosphotyrosine, PY-99 (Santa Cruz, CA), no sodium 
pyrophosphate was included in the lysis buffer.

**Immunoprecipitation and Western Blotting**—Freshly isolated monocytes were isolated and adhered to 6-well plates (5 × 10^6 cells/well) 
in the presence of BCS (10%) and DMEM for 2 h. The plates were washed once with fresh DMEM (without methionine) containing no BCS and 
subsequently replaced with DMEM (also without methionine) containing radiolabeled methionine (100 µCi/ml and 0.1% BCS) as 
well. The plates were incubated for 4 h and washed with fresh DMEM 
containing 10% BCS, and subsequently, the cells were lysed after 0, 4, 
8, 12, and 24 h of further incubations. Each one of the Jak/Tyk kinasas 
were immunoprecipitated from the lysates (10 × 10^6/g), run on a 
7.5% SDS-PAGE gel, transferred onto PVDF membranes, and exposed 
on a PhosphorImager screen (Molecular Dynamics). The half-life of the 
protein was calculated as the time necessary for the dpm of incorpo-
rated, radioactive methionine in that protein to decrease by 50%.

**Treatment of Monocytes with Oligodeoxyribonucleotides**—The anti-
sense sequences for human Jak2, Tyk2, and Jak1 were selected after 
studying the predicted secondary structural conformation of their 
mRNAs using the software program Mulfold©. The regions lacking 
major predicted secondary structures, e.g. loops, were mainly targeted 
to generate the antisense ODNs. Before final selection of the target 
region, the sequences were screened for uniqueness in all nonredundant 
GenBank CDS translations + PDB + SwissProt + PIR using Blast© 
and also were tested for lack of internal secondary structure or pairing 
using Mulfold© (48).

The antisense oligomer selected against Jak2 was complementary to nucleotides 59–78 of the human Jak2 sequence (accession number 
AA453345), whereas the antisense oligomer against Tyk2 was comple-
mentary to nucleotides 481–500 of the human Tyk2 sequence (accession 
number X54637). The antisense ODN to Jak1 was complementary to bases 
462–480 of the human Jak1 sequence (accession number NM61174).

Control ODN for Jak2 and Tyk2 consisted of sense ODN. The sense
IL-13-mediated 15-LO Induction Requires Continuous Exposure—Nassar et al. (21) studied the induction of 15-LO in response to constant exposure to IL-13 and found that the expression of 15-LO was not immediate and was detectable by Western blot after 36 h of incubation. To determine whether continuous exposure to IL-13 was required for 15-LO induction, we exposed monocytes to IL-13 (250 pg/ml) for various times, removed the IL-13 by washing, and then detected 15-LO expression at 36 h. The results (Fig. 1) revealed that only after 12 h of incubation with IL-13 was there detectable expression of 15-LO. The level of 15-LO expression was up-regulated when incubations were continued for 24 and 36 h. No 15-LO band was detected in the lysate of cells treated with IL-13 for less than 12 h or in the untreated cell lysate.

Numerous studies have reported the involvement of Jak/Tyk kinases and the STAT molecules in interleukin-mediated signaling pathways (23–26). At the same time, the reports indicate that these activation and tyrosine phosphorylation events take place immediately, within a few min of treatment. But in our case, we found that for the expression of 15-LO, the cells needed to be stimulated with the ligand (IL-13) for a minimum of 12 h. We were therefore interested in examining the role of Jak/Tyk involvement in 15-LO expression.

Effect of Kinase Inhibitors on IL-13-mediated Induction of 15-LO Expression—Previous studies have shown IL-13-mediated tyrosine phosphorylation of numerous proteins in human monocytes (50). To examine whether IL-13-mediated induction of 15-LO expression involved signaling by tyrosine phosphorylation events, we examined the effects of potent tyrosine kinase inhibitors on this pathway. First, the monocytes were pretreated with tyrosine kinase inhibitors like genistein and tyrphostin 23 for 30 min at different dosages followed by treatment with IL-13 for 24 h, and the level of 15-LO was studied by Western blotting (Fig. 2). Whereas 10 µg of tyrphostin 23/ml of medium caused only slight inhibition, 25 µg/ml had a more profound effect (~50% inhibition). At 50 µg/ml, tyrphostin 23 inhibited detectable 15-LO expression by approximately 98%. Genistein also substantially inhibited the induction of 15-LO when applied at 25 µg/ml. In contrast, the negative structural analog controls for both genistein (daidzein) and tyrphostin 23 (tyrphostin 1) had little effect (<10% inhibition) on the expression of 15-LO when tested at identical concentrations. These results indicate that both of the tyrosine kinase inhibitors were able to substantially inhibit IL-13-mediated induction of 15-LO.

IL-13 Induces Tyrosine Phosphorylation of Jak2 and Tyk2—To determine whether the Jak family of kinases was involved in IL-13-mediated signaling pathways, cell lysates from both untreated and IL-13-treated fresh human monocytes were immunoprecipitated with antibodies to Jak1, Jak2, Tyk2, and Jak3, electrophoresed, blotted on PVDF membranes, and immunoblotted with antibody to phosphotyrosine (Fig. 3A). We found that IL-13 induced phosphorylation of Tyk2 kinase within 5 min of treatment. Jak1 and Jak2 kinases were constitutively phosphorylated at low levels, and IL-13 treatment enhanced the level of phosphorylation of Jak2 considerably, whereas Jak1 phosphorylation was not induced. Monocytes expressed very low levels of Jak3, and no increased phosphorylation of Jak3 was observed in response to IL-13.

Phosphorylation of the Jak family of kinases was also studied by immunoprecipitating the tyrosine-phosphorylated proteins from untreated and IL-13-treated monocyte cell lysates using antibody to phosphotyrosine followed by blotting with different Jak/Tyk kinase antibodies. Data presented in Fig. 3B indicate that antibody to phosphotyrosine could immunoprecipitate both Jak2 and Tyk2 from the IL-13-treated cells, whereas no Tyk2 and a basal level of Jak2 were observed in the
lysates from each sample were loaded on each lane of a 7.5% SDS-PAGE gel, electrophoresed, blotted on PVDF membrane, and immunoblotted with the corresponding kinase antibodies. Fig. 4 shows the effect of treatment with antisense and sense ODNs designed to target Tyk2 in human monocytes. The results show that antisense ODNs to Tyk2 inhibited the expression of Tyk2 by 75–85% in the monocytes, whereas sense ODNs as well as the cationic lipid vehicle alone had no effect. The same blot was reprobed with antibody to Jak2 kinase after stripping to check the effect of antisense or sense ODNs to Tyk2 on the level of expression of Jak2 in monocytes. The bottom panel shows nearly equal levels of Jak2 protein in all the lanes, thus suggesting that the antisense oligomer for Tyk2 inhibited the expression of the target protein (Tyk2) while having no effect on the level of expression of Jak2.

Similar experiments were conducted using antisense ODNs against Jak2 (Fig. 5). Nearly 80% inhibition of Jak2 expression was noted when the monocytes were treated with antisense ODNs to Jak2. No such effect was observed when sense ODNs were used to treat the cells nor in cells treated with the cationic lipid vehicle. Stripping and reprobing experiments using Tyk2 kinase antibody to examine the effect of antisense Jak2 on the level of Tyk2 kinase expression showed that antisense to Jak2 specifically inhibited the level of expression of Jak2 while having no effect on Tyk2 expression.

For these studies, monocytes were treated with antisense or sense ODN against Jak2 or Tyk2 kinases for 24 h. After treatment, the cells were exposed to IL-13 for another 24 h. Finally the cells were lysed, run on SDS-PAGE, electroblotted, and probed with antibody directed against rabbit reticulocyte 15-LO, which cross-reacts with human 15-LO.

The results are shown in Fig. 6. The antisense ODN against either the Jak2 or Tyk2 kinases inhibited the IL-13-mediated induction of expression of 15-LO, whereas the sense ODN and the vehicle controls had no effect on 15-LO expression. As we have already established that the Jak2 antisense has no effect on the level of expression of Tyk2 and vice versa, our results clearly established that both Jak2 and Tyk2 were absolutely required for IL-13 induction of 15-LO expression.

### Diagrams

**Fig. 3.** Tyrosine phosphorylation is selectively enhanced after stimulation with IL-13 in the human monocytes. Human blood monocytes were treated with 250 μg of IL-13 for 5 min or left untreated as indicated. A, cells were lysed, the cleared cell lysates were immunoprecipitated (IP) with different Jak/Tyk antibodies, and the immune complexes were analyzed by immunoblotting with a mixture (1:1) of antibodies to phosphotyrosine, 4G-10 and PY-20. Arrows indicate the positions of respective kinases as mentioned, based on the migration of molecular weight markers that were run in adjacent wells. The bottom panel in each set represents reprobing with the same individual antibody used for immunoprecipitation, to indicate equal protein loading. B, cell lysates were immunoprecipitated with antibody to phosphotyrosine, PY-99, and the immune complexes were analyzed by immunoblotting with either Jak2 or Tyk2 antibody. Arrows indicate the positions of Jak2 (130 kDa) and Tyk2 (135 kDa) calculated from the migration of molecular weight markers in adjacent lanes.

**Fig. 4.** Expression of Tyk2 is inhibited by treatment with specific antisense oligodeoxyribonucleotides. Monocytes (5 × 10^6) were treated with antisense or sense ODNs (1.0 μM) to Tyk2 as indicated under “Methods.” After a 24-h incubation, cell lysates were prepared and loaded (50 μg or 100 μg) on a 7.5% SDS-PAGE gel, transferred, and immunoblotted with antibody to Tyk2. The arrow indicates the position of Tyk2 (135 kDa). The same blot was stripped and reprobed with antibody to Jak2 (130 kDa) as is shown in the bottom panel. The positions of the kinases were calculated based on the migration of molecular weight markers in adjacent lanes. The results are representative of at least three similar experiments.
necessary for the IL-13-mediated signaling, and depriving the monocytes of either of these enzymes caused the inhibition of expression of 15-LO.

Jak1 Is Not Involved in Regulating the Induction of 15-LO Expression in Response to IL-13—As an additional control, we also examined whether inhibition of Jak1 altered expression of 15-LO. As noted in Fig. 3A, Jak1, in contrast to Jak2 and Tyk2, was not phosphorylated on tyrosine in response to IL-13 treatment and therefore would not be predicted to regulate IL-13-mediated events. We prepared an antisense ODN to Jak1 and a scrambled ODN control. Monocytes were treated with antisense or control ODN or with the cationic lipid vehicle alone for 24 h as indicated under “Methods” and either lysed to check the level of Jak1 (Fig. 7A) or incubated an additional 24 h with IL-13 to examine induction of 15-LO expression (Fig. 7B). Results indicated substantial inhibition of Jak1 expression (−90%) in the monocytes treated with antisense to Jak1, whereas treatment with the control ODN or with the vehicle alone caused no inhibition of Jak1 expression (Fig. 7A). We then examined whether inhibition of Jak1 expression affected the IL-13-induced expression of 15-LO. Results displayed in Fig. 7B indicate that 15-LO expression was not inhibited in monocytes with lower levels of Jak1. In contrast, induction of 15-LO expression was substantially diminished by inhibition of either Jak2 or Tyk2 in confirmation of the results presented in Fig. 6.

DISCUSSION

IL-4 and IL-13 are the only cytokines that have been shown to induce both the expression and activity of 15-LO (20, 21). In both cases, a substantial increase in expression of 15-LO was shown, but the minimum exposure to cytokine required for the effect had not been examined. Our studies indicate that a minimum exposure to IL-13 of 12 h is sufficient to induce the expression of 15-LO (as detected at 36 h), but maximal expression was observed when IL-13 was present for the full 36-h period. The delayed expression of 15-LO may be the result of a complex signaling cascade and likely involves, because of the lengthy time frame, new transcription/translation of an activator or degradation of a protein inhibitor. Inhibition of induction of 15-LO in the IL-13-treated monocytes by potent tyrosine kinase inhibitors suggest that activation and phosphorylation of tyrosine kinases are necessary for 15-LO induction.

Several earlier reports have predicted that the receptors for interleukins are differentially regulated depending on the cell type studied (23–26). For example, although the level of expression of IL-4R was up-regulated in response to IL-4 in T-cells, IL-4 had no such effect on the IL-4R expression in monocytes (24). It is not quite well-established that IL-4R plays a role in the IL-13-mediated signaling in conjunction with another receptor subunit (IL-13R) (28). In T-cells, IL-2Rσc is suggested to be a member of IL-4 and IL-13 cytokine receptor superfamily, but the level of IL-2Rσc is extremely low (40) or absent (24) in human monocytes. Thus, Jak3, which has been shown to be associated with the IL-2Rσc receptor subunit in T- and B-cells and several other cell lines (51), seems not to serve as a signaling intermediate in the IL-13 responses of human mono-
cytes. Moreover, the level of Jak3 in unactivated monocytes has been shown to be extremely low and is induced upon monocyte activation following IL-2, LPS and IFN-γ treatment (51). Interestingly, IL-2Rγc expression also is up-regulated in activated monocytes, although nearly absent in freshly isolated monocytes (40). Thus, it seems possible that signaling intermediates in fresh, unactivated as compared with activated monocytes may vary depending upon the level of expression of both kinases and receptor molecules. In fact, Musso et al. (51) have shown that in LPS-activated monocytes, Jak3 is tyrosine-phosphorylated in response to IL-4; in contrast IL-4 did not induce tyrosine phosphorylation of Jak3 in unactivated monocytes. This finding is consistent with our results with IL-13 that clearly showed that phosphorylation of Jak3 was not augmented in fresh monocytes treated with IL-13. These data indicate the participation of tyrosine kinases other than Jak3 in IL-13-treated freshly isolated monocytes.

Our results indicate that Jak2 and Tyk2 were tyrosine-phosphorylated in response to IL-13. Although a basal level of phosphorylated Jak1 was detected, no induction of phosphorylation was observed in response to IL-13. There is an earlier report of Jak2 activation in response to IL-13 in a human ovarian carcinoma cell line (52). Jak1 and Tyk2 phosphorylation was also induced in these carcinoma cells. Jak2 was also phosphorylated in human endothelial cells in response to IL-13 (55). Another study on lymphohematopoietic cells (TF-1) showed that Tyk2 and Jak1 were tyrosine-phosphorylated in response to IL-13 (35), whereas IL-13-induced phosphorylation of Tyk2, but not Jak1 or Jak2, in an Epstein-Barr virus-transformed B cell line (53). In a recent study, Yu et al. (54) reported that NK cells and T cells respond to IL-13 by phosphorylating tyrosines on Jak3, and in murine plasmacytoma cell lines (B9), Tyk2 and Jak3 were shown to be phosphorylated when treated with either IL-4 or IL-13 (39). The disparity of these responses likely reflects the involvement of different IL-13 receptor components as well as variability in expression of the Jak kinases in these distinct cell types.

Further studies investigating the association of Jak2 and Tyk2 with the receptor molecules showed that Jak2 but not Tyk2 was associated and co-immunoprecipitated with the IL-13Rα subunit; thus, the association of Tyk2 with an IL-13R molecule is predicted. Moreover, although in some cells and cell lines an association between Jak1 and the IL-4Rα chain has been reported, we found that both Jak1 and Jak2 can associate with the IL-4Rα in the IL-13-treated human monocytes.2

To more specifically address the participation of Jak2 and Tyk2, both Jak2- and Tyk2-specific antisense and sense ODNs were developed. The sequences were carefully chosen from regions predicted to be without secondary structure and lacking substantial homology with other sequenced human mRNAs or genes. The oligonucleotides were phosphorothioate-modified to limit degradation and purified by HPLC before use to remove all incomplete synthesis products, thereby limiting nonspecific effects. We have found this latter step to be critical in rendering specificity to antisense ODN activity in human monocytes. We found by Western blot analysis that the antisense treatment, but not treatment with control, sense ODNs, resulted in decreased expression of both Jak2 and Tyk2. We also have established that the ODNs were very specific, showing that antisense ODN to Jak2 had no effect on the level of expression of Tyk2, and antisense ODN to Tyk2 did not inhibit Jak2 expression. The monocytes, pretreated with antisense ODN to either Jak2 or Tyk2, could not induce 15-LO expression when treated with IL-13 for 24 h, thereby leading us to believe that expression of both Jak2 and Tyk2 is absolutely necessary for the IL-13-mediated signaling pathway, resulting in the induction of expression of 15-LO. As per the proposed model of the mechanism of action of the Jak/STAT pathway (31), where Jak2 and Tyk2 would phosphorylate each other after ligand (IL-13)-mediated heterodimerization of the activated receptor molecules, our data shows that obliteration of either of the Jak/Tyk would abrogate the downstream effects in the IL-13-mediated signaling pathway.

We additionally induced the expression of a tyrosine kinase that was not activated by treatment of monocytes with IL-13, namely Jak1. Jak1 expression was substantially and selectively inhibited by treatment with antisense ODN to Jak1. The inhibition of Jak1 expression had no effect on the induction of 15-LO expression in response to IL-13 treatment. This finding was predicted because tyrosine phosphorylation of Jak1 was not induced by treatment with IL-13 and supports the involvement of select Jak kinases, namely Jak2 and Tyk2, in the induction of 15-LO expression by IL-13.

The results presented here suggest that IL-13-mediated induction of 15-LO expression in human monocytes needs a minimum stimulation of 12 h and requires tyrosine kinase activity. IL-13 treatment of the monocytes induced tyrosine phosphorylation of Jak2 and Tyk2, but not Jak1 or Jak3, within 5 min of treatment. Both of these tyrosine kinases appear to be required for the ultimate induction of 15-LO protein expression in IL-13-treated primary human monocytes.

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