Brief Definitive Report

IL-4 and IL-13 Induce Lsk, a Csk-like Tyrosine Kinase, in Human Monocytes

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Summary

Lsk is a protein tyrosine kinase with homology to the COOH-terminal Src kinase (Csk). Unlike Csk that is ubiquitously expressed, Lsk has limited tissue distribution. Here we have examined the expression and regulation of Lsk and Csk in peripheral human monocytes. We have found that Lsk mRNA and protein were not expressed in resting monocytes but were induced by treatment with interleukin 4 (IL-4) or IL-13 but not by interferon γ (IFN-γ) or IL-2. In fact, IFN-γ, but not IL-2, efficiently blocked Lsk induction by IL-4 or IL-13. In contrast, Csk was constitutively present in human monocytes and was upregulated by IFN-γ but not by IL-4 or IL-13. These results suggest that despite their structural similarities, Lsk and Csk may play distinct regulatory roles in monocyte functions elicited by cytokines, with Lsk functioning specifically within the context of a Th2-type immune response.

The phosphorylation of tyrosine residues by protein tyrosine kinases (PTKs) plays a central role in normal cellular growth and differentiation as well as oncogenesis. The 10 PTKs of the Src family (c-Src, c-Yes, Fyn, Yrk, c-Fgr, Lyn, Lck, Hck, Blk, and Rak) have been extensively studied. Whereas c-Src and c-Yes are widely expressed in tissues, other members of the Src family have a lineage-specific pattern of expression. For example, Lck is mainly expressed in T lymphocytes and natural killer cells and Blk is restricted to B cells. The principle Src-family PTKs in monocytes/macrophages are Hck, Fgr, Lyn, and Yrk; however, little is known about their function and regulation in these cells (1, 2). Nonetheless there is increasing evidence that tyrosine phosphorylation is important in monocytic functions. In monocytes, PTK activation has been shown to be required for the induction of cytokines by LPS (3) and Fc receptor–mediated phagocytosis (4, 5). In addition, both physical and functional association between Fc receptors and Src family PTK have been found (4, 5) and Fgr, Hck, and Lyn are regulated during macrophage activation by LPS and IFN-γ (6, 7).

The functions of Src-family PTKs are negatively regulated by phosphorylation of a highly conserved COOH-terminal tyrosine residue (Tyr 527 of Src) (8). COOH-terminal Src kinase (Csk), a ubiquitous nonreceptor tyrosine kinase, has been shown to be capable of specifically phosphorylating the negative regulatory sites of c-Src, Lck, Blk, and Fyn (9–11). Csk kinase differs from Src family members as it lacks myristoylation (Gly 2 of Src), and autophosphorylation sites, as well as the putative negative regulatory COOH-terminal tyrosine residue (11–13). These characteristics have suggested that Csk may be a member of a new class of nonreceptor PTKs involved in the downregulation of Src-family PTK kinase activity (12). We have recently cloned a highly related Csk-like gene, Lsk (leukocyte COOH-terminal Src-kinase) from activated peripheral blood T cells (14) and we have demonstrated the presence of abundant Lsk mRNA in natural killer cells and brain, with little or no expression in a variety of other tissues. In addition, several other groups have cloned genes highly homologous to Lsk (15–18).

This well-defined role of Src-family PTKs in monocyte/macrophage function in conjunction with the identification of a Csk-like kinase with preferential expression in the immune system led us to examine the regulation of the Csk family in normal human monocytes. We report here that although Lsk mRNA and protein were not constitutively expressed in untreated monocytes, the kinase was induced after treatment of monocytes with IL-4 or IL-13 and that induction was blocked by IFN-γ. In sharp contrast, Csk mRNA and protein were constitutively present in human monocytes and were upregulated by IFN-γ but not IL-4 or IL-13. This suggests that the expression of Lsk is highly regulated in monocytes by cytokines invoked during a Th2-type immune response, perhaps implying a specific role for Lsk in monocytes during these responses.

Materials and Methods

Cell Culture. Monocytes were purified from PBMC by centrifugal elutriation as described elsewhere (19). The purity of the monocyte preparations used in these study was 93% ± 3% as assessed by morphology on Giemsa-stained cytocentrifuge preparations and flow cytometry using the monocyte-specific mAb, LeuM3. Cells were cultured in RPMI 1640 (Advanced Biotechnologies, Inc., Columbia, MD), containing 100 U/ml penicillin, 100 μg/ml streptomycin, 2 mmol/liter L-glutamine, 20 mmol/liter Hepes (Gibco BRL, Gaithersburg, MD); and 10% heat-inactivated fetal bovine serum (HyClone Laboratories, Inc., Logan, UT).

Cytokines. Human recombinant IFN-γ (lot NN9027AX, sp act 2.02 × 10^7 U/mg) was kindly provided by Dr. H. M. Shephard (Genentech, San Francisco, CA). Human recombinant IL-4 was obtained from Pepro Tech (Rocky Hill, NJ). Human recombinant IL-13 was kindly provided by Sanofi Elf Bio Recherche (Labège, France; 0.2–0.5 ng/ml of Chinese hamster ovary–derived IL-13 supports 50% maximum proliferation of the TF-1 cell line). Recombinant purified IL-2 from *Escherichia coli* (lot LP-381, sp act of 18 × 10^6 IU/mg, and LPS content of <0.0006 ng/ml) was kindly provided by Cetus Corp. (Emeryville, CA).

Northern Blot Analysis. For RNA extraction, 5 × 10^7 monocytes were plated in 25 ml medium alone or supplemented with the indicated cytokines. Total mRNA isolation and Northern blot analysis were performed as described (19). mRNA expression was quantitated by digital scanning of autoradiographs and the values reported are the average ± standard deviation where the control expression is represented as 1.0.

Western Blot Analysis. 50 × 10^6 monocytes were cultured in medium alone or in the presence of the indicated cytokines for 48 h. Cells were washed in PBS and lysed by boiling in Laemmli sample buffer. Insoluble material was removed by centrifugation for 30 min at 10,000 *g*, protein content of the clarified lysate was determined using a protein assay kit (Bio-Rad Laboratories, Richmond, CA) and equal amounts of protein from each sample were subjected to SDS-PAGE, and immunoblotted with a polyclonal rabbit serum generated to the predicted COOH-terminal of the Lsk protein (14) or with polyclonal anti-Csk antiserum (kind gift of Dr. M. Okada, Osaka, Japan) as described (14).

Results

Differential Regulation of Lsk and Csk in Human Monocytes. IFN-γ is a potent activator of several human monocyte functions. Many of the monocyte/macrophage activities induced by IFN-γ as well as other monocyte activators can be downregulated by IL-4. In addition, IL-4 induces the expression of certain monocyte/macrophage surface antigens including MHC class II molecules and CD23 (20). To establish if IFN-γ or IL-4 could regulate Lsk expression, total RNA was obtained from monocytes cultured for 18 h with medium alone or supplemented with IL-4 (100 U/ml), IFN-γ (500 U/ml), or IL-4 (100 U/ml) plus IFN-γ (500 U/ml). As shown in Fig. 1 *A*, Lsk mRNA was not constitutively expressed in untreated monocytes. Interestingly, the monocyte activator IFN-γ failed to induce significant Lsk mRNA increase compared to unstimulated monocytes (1.17 ± 0.26 times the level of the medium control, $n = 5$) (Fig. 1 *A*, lane *3*) as did LPS, IL-1, and TNF (data not shown). In contrast, treatment of monocytes with IL-4 induced a pronounced increase of Lsk mRNA (9.42 ± 2.7 times the level of the medium control, $n = 5$) (Fig. 1 *A*, lane *2*). Time course experiments indicated that Lsk mRNA expression was observed as early as 6 h after IL-4 stimulation, was maximal at 18 h, and remained elevated for up to 48 h (data not shown). Coculture with IFN-γ (500 U/ml) completely inhibited the induction of Lsk by IL-4 (1.31 ± 0.23 times the level of the medium control, $n = 5$) (Fig. 1 *A*, lane *4*). As shown in Fig. 2, *A* and *B*, IL-4 induction of Lsk mRNA and its inhibition by IFN-γ were dose dependent. Since IL-2, like IFN-γ, stimulates several monocytic functions (19, 21) we investigated the effect of IL-2 on Lsk mRNA expression. Interestingly, IL-2 did not induce Lsk mRNA and, in contrast to IFN-γ, did not affect IL-4 induction of Lsk. These data suggest that Lsk expression in human monocytes is tightly regulated by IL-4 and IFN-γ.

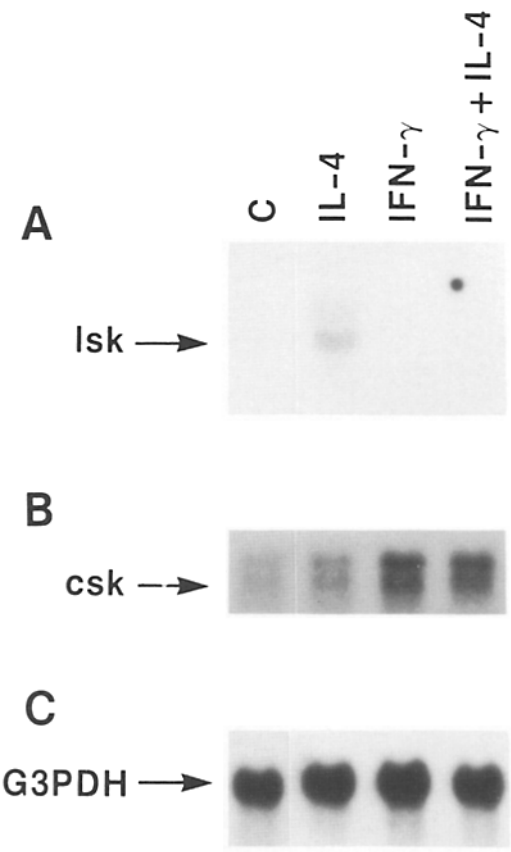

Figure 1. Effect of IL-4 and IFN-γ on Lsk and Csk expression. Northern blot analysis of the expression of Lsk mRNA (*A*) or Csk mRNA (*B*) in human monocytes cultured for 18 h with medium alone (lane *1*); IL-4, 100 U/ml (lane *2*); IFN-γ, 500 U/ml (lane *3*); and IFN-γ plus IL-4 (lane *4*). Expression of G3PDH (*C*) demonstrated that equal amounts of RNA were loaded in each lane.

Because together Lsk and Csk comprise a unique family of kinases, we next analyzed the expression of Csk in IFN-γ– and IL-4–stimulated monocytes. We found that Csk mRNA was constitutively expressed in human monocytes (Fig. 1 *B*) and unaffected by IL-4 treatment (1.18 ± 0.07 times the levels of control, $n = 3$). While IFN-γ did not induce Lsk expression in monocytes, Csk mRNA was slightly increased after IFN-γ treatment (1.54 ± 0.24 times the level of control,

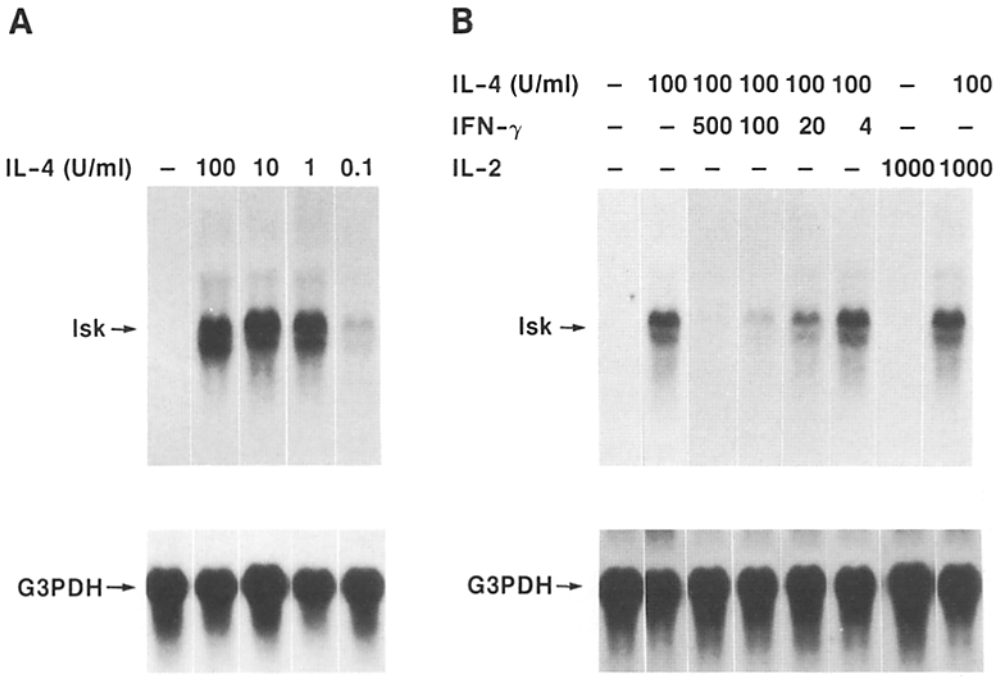

Figure 2. Dose-dependent induction of Lsk mRNA by IL-4. Monocytes were cultured for 18 h in the presence of medium alone (lane *1*) or decreasing concentrations of IL-4, 100 to 0.1 U/ml (lanes *2–5*). The same filter was subsequently rehybridized with a probe specific for G3PDH to demonstrate that equal amounts of RNA were loaded. (*B*) IL-4–induced expression of Lsk in monocytes is antagonized by IFN-γ but not by IL-2. Monocytes were cultured for 18 h in the presence of medium alone (lane *1*); IL-4, 100 U/ml (lane *2*), or IL-4 plus decreasing concentration of IFN-γ, 500 to 4 U/ml (lanes *3–6*); IL-2, 1,000 U/ml (lane *7*) or IL-2 plus 100 U/ml IL-4 (lane *8*). The same filter was subsequently rehybridized with a probe specific for G3PDH to demonstrate equal RNA loading.

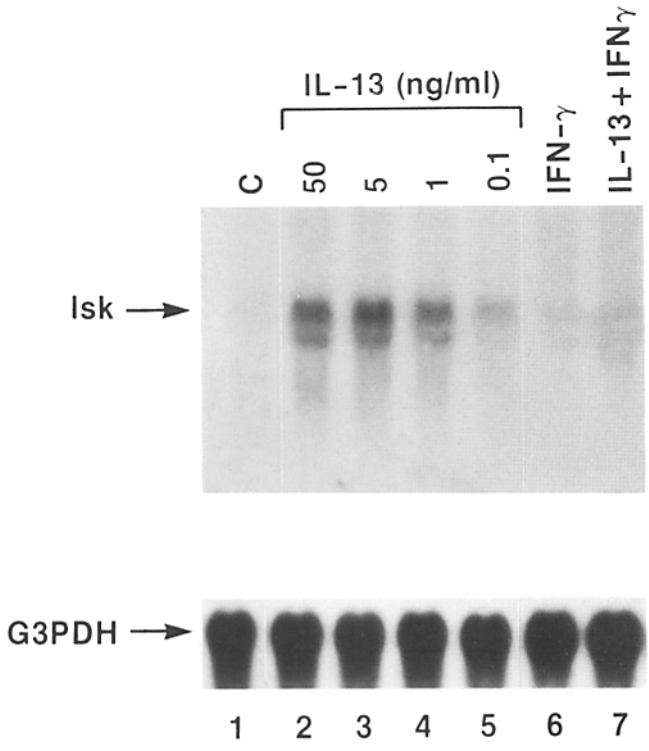

Figure 3. Effect of IL-13 and IFN-γ on Lsk mRNA expression. Monocytes were cultured for 18 h in the presence of medium alone (lane *1*); decreasing concentrations of IL-13, 50 to 0.1 ng/ml (lanes *2–5*); IFN-γ, 500 U/ml (lane *6*); or IL-13, 50 ng/ml plus IFN-γ, 500 U/ml (lane *7*). Expression of G3PDH demonstrated equal RNA loading.

n = 3). Therefore, the cytokine regulation of Lsk and Csk differs substantially in monocytes.

Effect of IL-13 on Lsk mRNA. A recently identified cytokine, IL-13, has been shown to share some of the activities of IL-4 (22, 23). Therefore, we examined whether IL-13 might also upregulate Lsk gene expression. Dose response experiments were performed in monocytes stimulated for 18 h by IL-13. Fig. 3 shows that IL-13 induced Lsk mRNA in a dose-dependent fashion (IL-13 at 50 ng/ml increased Lsk mRNA to 3.7 ± 0.28 times the level of medium alone, n = 2). Since Lsk induction by IL-4 was inhibited by IFN-γ, we examined its effect on IL-13-induction of Lsk. As shown in Fig. 3, IFN-γ completely blocked the induction of Lsk by IL-13. These results demonstrated that IL-13 induces Lsk mRNA in human monocytes and, like IL-4, IL-13 effects are inhibited by IFN-γ.

Induction of p57^{Lsk} in IL-4– and IL-13–treated Monocytes. We next sought to verify whether Lsk mRNA upregulation also results in an induction of p57^{Lsk}, the protein encoded by the Lsk gene. To this end, Western blot analysis was performed using an anti-Lsk polyclonal rabbit antiserum. Fig. 4 shows that a polypeptide of ~57 kD corresponding to p57^{Lsk} was detected in total cell lysates of monocytes treated with IL-4 (*A*) or IL-13 (*B*) for 48 h, but not in the untreated cells. Monocytes treated for 48 h with IFN-γ, IFN-γ plus IL-4, or IFN-γ plus IL-13 did not possess detectable levels of p57^{Lsk}. The specificity of the Lsk antiserum was demonstrated by the ability of the Lsk peptide to compete the binding of antiserum to the 57-kD polypeptide but not the 69- or 52-kD nonspecific bands (data not shown) (14). Using the anti-Csk antiserum we analyzed the effect of IL-4, IL-13, IFN-γ treatments on levels of p50^{Csk}. Fig. 5 shows that consistent with the results of the Northern analysis, p50^{Csk} was

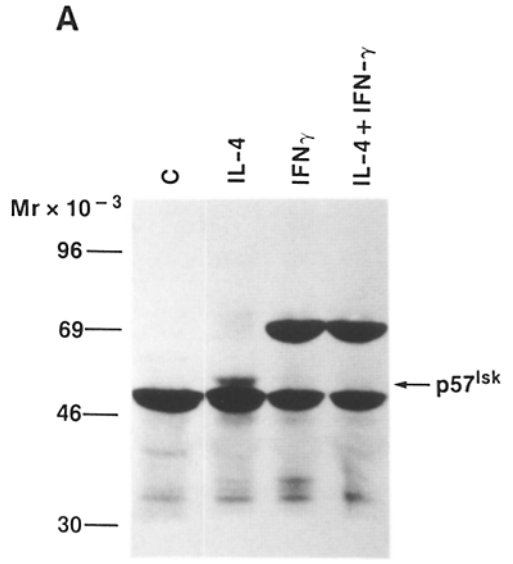

Figure 4. $p57^{lsk}$ expression in human monocytes. Monocytes were treated for 48 h with medium alone (*C*); IL-4, 100 U/ml; IFN-γ, 500 U/ml; IL-4 plus IFN-γ (*A*) or IL-13, 50 ng/ml, or IL-13 plus IFN-γ (*B*) were lysed and postnuclear supernatants were electrophoresed and immunoblotted using a polyclonal rabbit antiserum generated against the COOH terminus of Lsk.

present in untreated cells, unaffected by IL-4 or IL-13, and slightly increased by IFN-γ treatment.

Discussion

The Lsk and Csk genes encode structurally related PTKs (11, 13, 14). The established role of Csk suggests that both kinases may negatively regulate the enzymatic activity of Src family members involved in leukocyte activation (9, 11, 17, 18). However, the fact that Csk is ubiquitously expressed whereas Lsk expression is more limited, indicates differential regulation of Csk family members and implies diverse functions for the different family members. Here we report that Lsk is not constitutively expressed in human monocytes, but is induced by IL-4 or IL-13 treatment. IFN-γ, a potent monocyte activator, did not induce Lsk; rather, it inhibited IL-4 or IL-13 induction. In contrast, Csk is constitutively expressed in human monocytes, not affected by IL-4 or IL-13 treatment, and upregulated slightly by IFN-γ.

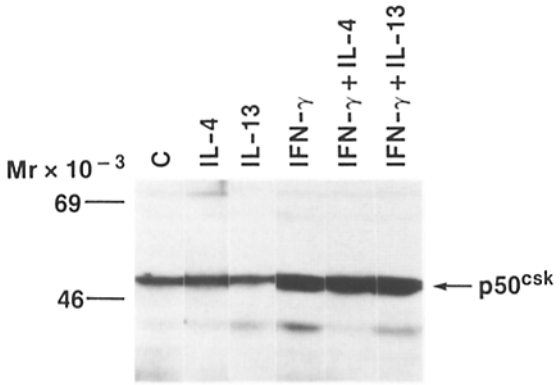

Figure 5. $p50^{csk}$ expression in human monocytes. Monocytes were treated for 48 h with medium (*C*); IL-4, 100 U/ml; IL-13, 50 ng/ml; IFN-γ, 500 U/ml; IL-4 plus IFN-γ, or IL-13 plus IFN-γ, lysed, and postnuclear supernatants were electrophoresed and immunoblotted using an anti-Csk antiserum.

IFN-γ and IL-4 are produced by functionally different subsets of T helper cells, Th1 and Th2, respectively. IFN-γ–producing T clones have been shown to direct the immune response towards cytotoxicity by activating monocytes and promoting cytotoxic T cell activity. In contrast, IL-4–secreting T cells preferentially augment a humoral immune response by inducing B cell activation and inhibiting monocytic activities (24). Therefore, IL-4 and IFN-γ, as products of two distinct subsets of T helper lymphocytes, control the state of activation of monocytes. Our data suggest that Lsk regulation by IL-4 and IFN-γ may play a role in the modulation of monocyte functions by T helper subsets.

In addition to IFN-γ, the Th1 phenotype is characterized by IL-2 production. We have previously shown that like IFN-γ, IL-2 can activate human monocyte cytotoxicity (25, 26) and increase the expression of several monocyte genes (19, 21, 25, 27). However, IL-4 blocks IL-2–induced but not IFN-γ–induced monocyte cytotoxicity (Bosco, M., K. Pulkki, A. Zea, T. Musso, D. L. Longo, L. Varesio, and I. Espinoza-Delgado, manuscript in preparation). Since our data show that IL-4 is unable to induce Lsk in the presence of IFN-γ, but does in the presence of IL-2, IL-4 might inhibit certain monocyte activities, such as cytotoxicity, in part through the induction of Lsk.

Unlike the Src family PTK, to date there is only one report of modulation of Lsk or Csk enzymatic activity (28). Therefore, the cytokine-mediated transcriptional/translational control we have demonstrated here may represent a principle mechanism by which the Csk family is regulated. However, the differential regulation in human monocytes of these two Csk-family genes is intriguing. The fact that Csk is constitutively present in monocytes is not surprising since Csk has been shown to be expressed in all human tissues and cell lines tested to date (11–13). However, the observation that Csk expression can be regulated in human monocytes by cytokines has not been previously reported. The constitutive expression of Csk and its mild upregulation by IFN-γ, in conjunction with the reported upregulation of Fgr, Hck, and Lyn

by IFN-γ and/or LPS (6, 7) in monocytes, suggest that Csk may function as a general regulator of several Src-family kinase activities. Lsk, on the contrary, could preferentially phosphorylate certain Src family members or other substrates involved in the differential effects of IL-4 and IFN-γ in monocytes. This hypothesis is in part supported by structural differences in the SH3 domains of Lsk and Csk indicating different intracellular localizations and/or substrates for these kinases (29).

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