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A 70-KDa Protein Facilitates Interleukin-4 Signal Transduction in the Absence of the Common Gamma Receptor Chain

C. H. Dawson, B. L. Brown,* and P. R. M. Dobson[1]
*Institute for Cancer Studies and *Department of Human Metabolism and Clinical Biochemistry, University of Sheffield Medical School, Beech Hill Road, Sheffield, S10 2RX, United Kingdom*

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Interleukin-4 signal transduction (and activation of STAT 6) is known to be mediated via its binding to a p140 receptor chain and the common gamma chain (γc). In non-activated monocytes, neither the γc nor its associated signal transducing molecule, Jak3, is expressed. We nevertheless show that IL-4 can initiate the tyrosine phosphorylation and DNA binding of STAT 6 in these cells. We present evidence for an additional 70 kDa IL-4 receptor chain which mediates the tyrosine phosphorylation of STAT 6 via Jak2, and suggest that this is the means by which IL-4 can signal in cells lacking the γc.

The T-cell-derived cytokine, interleukin-4 (IL-4), initiates tyrosine phosphorylation of the most recently described member of the STAT (Signal Transducers and Activators of Transcription) family, STAT 6. As a result of tyrosine phosphorylation, STAT 6, like other STAT factors translocates to the nucleus where it induces gene transcription by binding to a consensus site in the promoter regions of responsive genes. The best documented of the genes bound by STAT 6 are those for IgE, and the low affinity IgE receptor, CD23b/FceRII (1-3). Prior to activation of STAT 6, IL-4 binds to a cell surface receptor comprised of a 140 kDa chain, which it shares with IL-13 (4), and the γc which is also a component of receptors for IL-2, -7, -9, -12, and -15 (5, 6). Ligand binding binding of the 140 kDa chain has been reported to initiate Jak1 tyrosine phosphorylation (7), while binding to the γc results in Jak3 activation (8). Jak3 and Jak 1 are activated by reciprocal tyrosine phosphorylation, and subsequently activate STAT factors including STAT 6 (8).

Despite undisputed evidence showing the γc to be a functional component of the IL-4 receptor, we show in this report that in the monocytic cell line, U937, IL-4 can initiate STAT 6 tyrosine phosphorylation and DNA binding activity in the absence of γc expression. Furthermore, STAT 6 activity is not dependent upon Jak3. We present evidence for a 70 kDa protein which may be a third IL-4 receptor component which associates with Jak2 to mediate tyrosine phosphorylation of STAT 6, thus facilitating IL-4 responsiveness in cells which do not express the γc.

MATERIALS AND METHODS

Materials. Recombinant human IL-4 was a gift from Sandoz, Berne, Switzerland. Anti-IL-4 receptor antibody was a gift from Immunex Research Corp., Seattle, USA and TUGm2 was a gift from Professor K. Sugamura, Tohuku University, Japan. Anti-STAT 6 was purchased from Transduction Laboratories, Nottingham, UK, and all other antibodies were from UBI, Lake Placid, USA. Oligonucleotides were synthesised by Sheffield Molecular Synthesis, Sheffield, UK.

Cell culture and sample preparation. All cells were cultured according to standard techniques. Suspension cell lines (U937, WIL2NS, and H9) were grown in RPMI medium (Gibco/BRL, Paisley, Scotland, UK) supplemented with 10% v/v Foetal Calf Serum (Gibco/BRL) and 2mM L-glutamine. Adherent cells (T47D) were grown in DMEM (Gibco/BRL) supplemented similarly. Prior to addition of IL-4, cells were incubated in serum free medium containing 0.1% BSA and where indicated, stimulated for 24 hours with LPS (50ng/ml). Whole cell lysates were prepared by brief sonication of cells in buffer containing 20mM HEPES pH 7.4, 30mM p-nitrophenolphosphate, 10mM NaF, 10mM $MgCl_2$, 2mM EGTA, 6mM DTT, 0.2mM sodium orthovanadate, 2mM PMSF, 5mM leupeptin, 5mM pepstatin A, and 1% NP-40. Protein extracts were clarified by centrifugation at 13,000g. Nuclear extracts were prepared according to the method of Chan and Aggarwal (9). All samples were assayed for protein content according to the method of Bradford (10).

Immunoprecipitation and Western blotting. Samples were precleared by incubation for 1-2 hours at 4°C with Protein A agarose beads, followed by incubation overnight at 4°C with antibody-conjugated Protein A agarose beads. Immunoprecipitated proteins were resolved on a 10% SDS-PAGE gel and transferred to nitrocellulose

[1] To whom correspondence should be addressed.

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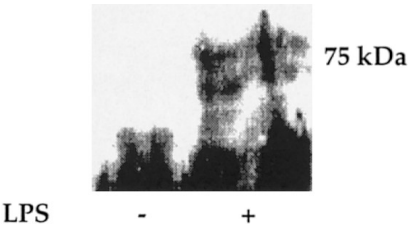

FIG. 1. U937 cells were serum-starved for 24 hours and stimulated for 24 hours with LPS (lane 2). Whole cell lysates were resolved by SDS-PAGE and blotted with TUGm2 antiserum against γc.

membrane. Following immunoblotting, proteins were visualised by ECL (Amersham Life Science, Little Chalfont, UK).

Electromobility shift analysis (EMSA). This was performed as described by Kohler and Rieber (2) using 6mg of nuclear protein extract/well. Double stranded oligonucleotides containing consensus STAT 6 binding sites in the IgE and CD23b promoters respectively read: 5′- tcgtAGTCTTCCCAAGAATGCATGACAC -3′, and 5′ - tcgtTCGATTCCTTTCTAAGAA CACCGAT -3′. STAT 6 binding sites are underlined.

RESULTS

Expression of the γc has been shown to be restricted to cells associated with the immune system, most notably B-cells, and T-cells (11). In monocytes, γc is expressed only in cells activated with agonists such as LPS for example (12). In support of this, Fig. 1 shows that γc expression, indicated by cross-reactivity of a 75 kDa protein with the anti-γc antiserum, TUGm2 (6, 13), was only detectable in U937 cells stimulated for 24 hours with LPS.

The tyrosine kinase, Jak3, is unique amongst members of its family in that its association with cytokine receptors is restricted to interaction with the γc. Consequently, only ligands binding the gc activate Jak3. As expected therefore, Fig. 2 shows that IL-4 did not activate Jak3 in the absence of the gc (in non-LPS-activated U937 cells), but initiated its tyrosine phosphorylation in LPS-activated cells. The 100 kDa protein may have been STAT 6. Despite reports to the contrary (7), we were unable to detect Jak1 activity in response to IL-4 in this cell line.

Previously published data (eg. 7, 8) has shown that Jak3 (and possibly Jak1) activity is a pre-requisite to STAT 6 tyrosine phosphorylation. However, despite the absence of Jak1 and Jak3 activity in non LPS activated cells, IL-4 initiated tyrosine phosphorylation (Fig. 3a) and DNA binding (Figs. 3b and 3c) of STAT 6. Activating the cells with LPS prior to stimulation with IL-4 increased the intensity of the signal.

Given the oligomeric nature of functional cytokine receptors, and the ability of IL-4 to activate STAT 6 independently of the γc, we reasoned that IL-4 may be able to recruit an alternative receptor component to facilitate signal transduction in the absence of the γc. Fig. 4a shows that the IL-4 receptor antibody used in our experiments also recognised a 70-80 kDa protein in both LPS-activated, and non-activated U937 cells. Furthermore, expression of this protein was not restricted to U937 cells, but was also detected in the B-cell line, WIL2NS, and the human epithelial breast cancer cell line, T47D. The T-cell line, H9, did not however express the 70-80 kDa protein.

In non-LPS-activated cells this protein was tyrosine phosphorylated upon ligand binding (Fig. 4b, lanes 1-4), suggesting that it may be a functional component of the IL-4 receptor. In LPS-activated cells, the putative 70-80 kDa receptor protein was constitutively tyrosine phosphorylated (Fig. 4b, lanes 5-8). The IL-4-induced activation of Jak2 (Fig. 5a) by recruitment of the kinase to the 70-80 kDa receptor chain (Fig. 5b) provides further evidence that the 70-80 kDa protein is a functional component of the IL-4 receptor. Jak2 also associated with STAT 6 (Fig. 5c), and may therefore be capable of mediating its tyrosine phosphorylation. In LPS-activated cells, Jak2 was constitutively active (Fig. 5a).

DISCUSSION

The evidence presented here for an additional IL-4 receptor subunit explains the γc-independent activation of STAT 6 and supports previous observations indicating that the gc is dispensible for many IL-4-induced (STAT 6-mediated) signalling events, including B-cell activation, proliferation, and IgE synthesis (14). Furthermore, our finding that Jak2 is recruited to the p70-80 receptor subunit supports recent evidence (15, 16) showing that IL-4 has the capacity to activate all known members of the Jak family. In view of the fact that Jaks are non-selective in their activation of STAT proteins (17), the data here showing interaction between Jak2 and STAT 6 suggests that Jak2 can mediate STAT 6 tyrosine phosphorylation. This may adequately explain the apparent dispensibility of Jak3 for STAT 6 tyrosine phosphorylation.

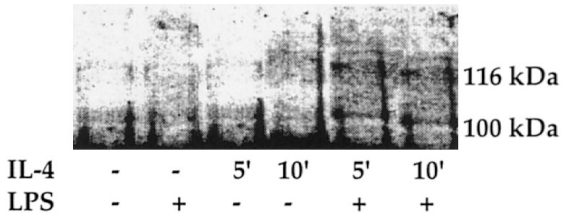

FIG. 2. Jak3 activity in U937 cells. Whole cell lysates were immunoprecipitated with anti-Jak3 and blotted with anti-phosphotyrosine. In lanes 4–6, cells were activated with LPS prior to stimulation with IL-4.

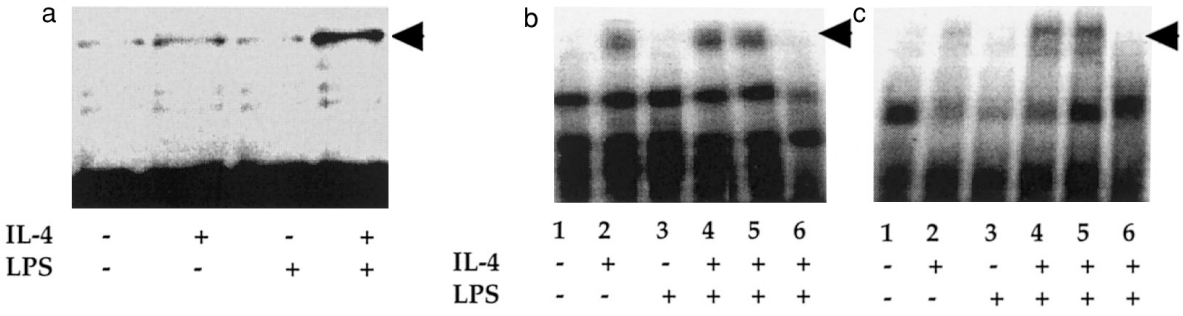

FIG. 3. STAT 6 activity in U937 cells. Whole cell lysates from cells stimulated with IL-4 and/or LPS were immunoprecipitated on anti-phosphotyrosine and blotted with anti-STAT 6 (a). Nuclear extracts from cells stimulated for the indicated times with IL-4 and/or LPS were incubated with a radiolabelled oligonucleotide containing the STAT 6 site in the IgE promoter (b) or the CD23b promoter (c). To check for binding specificity, the radiolabelled probe was competed for with a 50-fold molar excess of an unlabelled, unrelated oligonucleotide (lane 5 in (b) and (c)), or a 50-fold molar excess of an unlabelled target oligonucleotide (lane 6 in (b) and (c)).

This alternative (γc/Jak3-independent) route to STAT 6 activity is further supported by emerging evidence regarding the mechanisms of IL-13 signal transduction. IL-13 binds the p140 IL-4 receptor subunit and activates STAT 6, not by recruiting the γc or Jak3 (18), but instead by binding a 70 kDa receptor component in addition to the p140 subunit (4, 19, 20). IL-13 cannot however, signal in T-cells since they do not express this 70 kDa IL-13 receptor subunit (20). Interestingly, in our results, only the T-cell line, H9, failed to express the 70-80 kDa protein recognised by the IL-4 receptor antibody suggesting that the alternative IL-4 receptor component identified here may be the 70-80 kDa receptor chain bound by IL-13. Our findings support several recent reports showing that IL-4 and IL-13 compete for binding to receptor subunits of both 70 kDa and 140 kDa (18, 20). The sharing of peptides between the two proteins (21) presumably explains the cross-reactivity to both proteins of the IL-4 receptor antibody used in our experiments.

It is not possible from our data to accurately assess the role of the 70 kDa receptor subunit in cells expressing the γc. In view of the greater STAT 6 activity in LPS-activated U937 cells, it is tempting to envisage a trimeric IL-4 receptor arrangement analagous to the high affinity IL-2 receptor (22). Thus in cells which do not express the γc, such as resting monocytes and cells with a non-immune function, IL-4 utilises the p140 and p70 chains, and in T-cells which do not express the p70 subunit, IL-4 signals via p140 and the γc. However, in B-cells and activated monocytes which express all three, a high affinity trimeric IL-4 receptor may facilitate a greater response to IL-4.

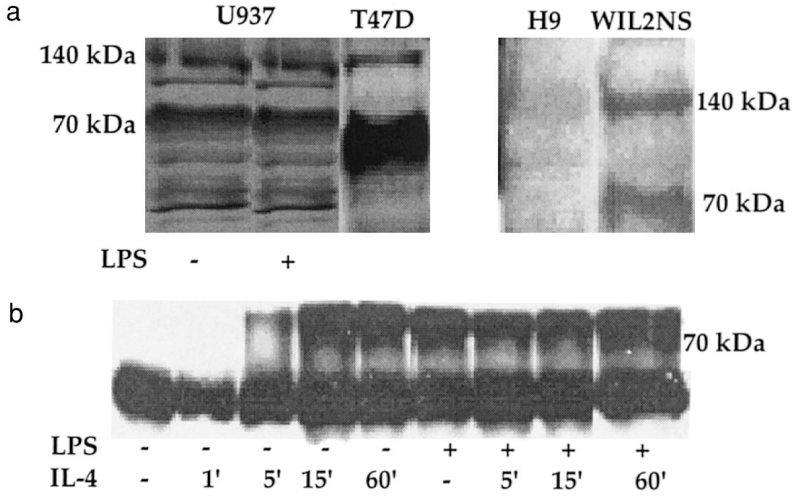

FIG. 4. IL-4 receptor expression. Whole cell extracts from a range of cell types (see text for details) were resolved by SDS-PAGE and blotted with anti-IL-4 receptor antibody (a). In (b), samples were immunoprecipitated with anti-phosphotyrosine before blotting with anti-IL-4 receptor antibody. Where indicated, cells were activated with LPS prior to stimulation with IL-4.

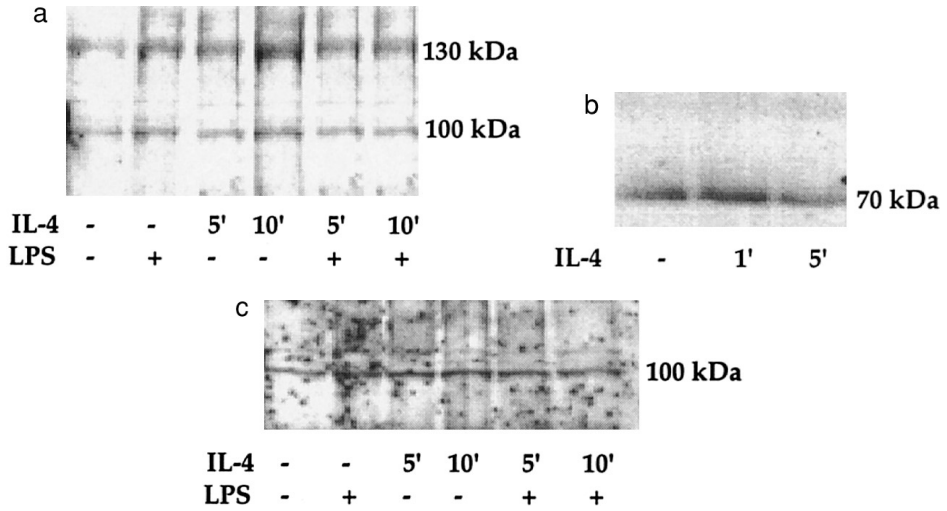

FIG. 5. IL-4-induced Jak2 activity in U937 cells. Whole cell lysates were immunoprecipitated on anti-Jak2, and blotted with anti-phosphotyrosine (a), anti-IL-4 receptor antibody (b), or anti-STAT 6 (c).

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