not shown). Consistent with previous results[11], Jak-3 is expressed in breast-tissue-derived cell lines (K.S.L. and E.T.L., unpublished observations).

To assess Jak-3 in signalling, cytokine-induced tyrosine phosphorylation was examined. Phosphorylation was not seen with erythropoietin, IL-3, granulocyte macrophage colony-stimulating factor (GM-CSF), G-CSF, interferon-α (IFN-α), IFN-γ or IL-6 (data not shown), but was seen in IL-2- or IL-4-stimulated cells. In CTLL cells, IL-2 and IL-4 induced tyrosine phosphorylation of several proteins (Fig. 4), including proteins of 120K and 130K, as recently shown[16]. IL-2 and IL-4 induced tyrosine phosphorylation of Jak-3 (αJak-3), which comigrated with the major 120K substrate, and Jak-1 (αJak-1), which comigrated with the 130K substrate. No Jak-2 or Tyk-2 phosphorylation was detected (data not shown). Last, the Jak-3/Jak-1-crossreactive Tyk-2 antiserum immunoprecipitated proteins that comigrated with Jak-1 and Jak-3 but not Tyk-2. Phosphorylation was detectable within 1 min, peaked at 20–30 min and subsequently declined (data not shown).

Cytokine-induced tyrosine phosphorylation of Jaks activates their *in vitro* kinase activity[1,2,4,7,9]. Therefore, we examined the effects of IL-2 or IL-4 on kinase activity. No kinase activity was detected in immunoprecipitates of Jak-1 or immunoprecipitates of Jak-3 obtained with the Jak-3-specific antiserum (data not shown). However, the Jak-3 antiserum is against a peptide containing the autophosphorylation site (KDYY; single-letter amino-acid code) and could interfere with activity. Therefore immunoprecipitates obtained with the Jak-1/Jak-3 crossreactive antiserum were examined. With these, IL-2- and IL-4-induced *in vitro* kinase activity was readily detected (Fig. 4*b*) and the single phosphorylated protein comigrated with Jak-3. No detectable Jak-1 phoshorylation was seen. Amino-acid analysis of phosphorylated Jak-3 detected exclusively phosphotyrosine (data not shown).

To assess the specificity of Jak-3 activation, we examined CTLL cells expressing the erythropoietin receptor, which shares homology in the cytoplasmic domain with the IL-2 receptor β-chain[17]. Transfected CTLL cells express levels of high-affinity erythropoietin receptors comparable to transfected myeloid cells (O.M. and J.N.I, unpublished data). Erythropoietin, but neither IL-2 nor IL-4, induced tyrosine phosphorylation of Jak-2 (Fig. 4*c*). Conversely, IL-2, but not erythropoietin, induced phosphorylation of Jak-3.

The IL-2 receptor consists of α-, β- and γ-chains[18]. The γ-chain is also used in the IL-4 receptor[19,20] and is essential for function. The β-chain is also essential and contains two functional domains. An acidic domain is required for association and activation of $p56^{lck}$, $p59^{fyn}$ or $p53/56^{lyn}$[18] but is dispensable for mitogenesis. In contrast, the membrane-proximal, serine-rich domain is required for mitogenesis. To assess their role in Jak-3 activation, the wild-type and receptors lacking the acidic (A-mutant) or serine-rich (S-mutant) domains were introduced into IL-3-dependent 32Dcl3 (Fig. 4*d*, *e*). Cells expressing the wild-type receptor, or the A-mutant, responded to IL-2 but not cells expressing the S-mutant. IL-3 stimulation of cells expressing the wild-type receptor resulted in only Jak-2 phosphorylation. In contrast, IL-2 stimulation induced Jak-3 phosphorylation, but not Jak-2 or Tyk-2, and activated Jak-3 *in vitro* kinase activity (data not shown). Of note, Jak-1 was not detectably phosphorylated in any of these experiments. Last, IL-2 stimulation of cells expressing the A-mutant-, but not the S-mutant-, induced activation of Jak-3.

The results demonstrate that IL-2 and IL-4 consistently activate Jak-3 and, in CTLL cells, also activate Jak-1. Activation of two Jaks is required for IFN signalling[7,8]; thus, it will be important to determine whether activation of both Jak-1 and Jak-3 is required for a T-cell response. Activation of Jak-3 requires the membrane-proximal, serine-rich region of the IL-2 receptor β-chain and thus correlates with mitogenesis. However, additional approaches are needed to determine if Jak-3 is essential for IL-2 signalling. In several cytokine receptors, the membrane-proximal, box1/box2 motifs bind Jaks. The presence of this motif in the IL-2 β-chain suggests that Jak-3 associates with the β-chain. Consistent with this, Jak-3 co-immunoprecipitates with the IL-2R complex (Johnston *et al.*, accompanying manuscript)[30] and previous studies[16] identified an IL-2-induced tyrosine phosphorylated 116K protein crosslinked to the β-chain which might be Jak-3. In addition to Jaks, cytokines activate transcription factors of the signal transducers and activators of transcription (STAT) family[21–25] and related proteins are induced by IL-4 and IL-2[26] (W. Thierfelder and J.N.I., unpublished data). Thus, the identification of Jak-3 in IL-2 and IL-4 signalling supports the general concept that cytokine receptor superfamily members couple ligand binding to activation of Jaks which, in turn, activate STATs as a primary signalling pathway. □

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CORRECTION

Binding of phosphatidylinositol-3-OH kinase to CD28 is required for T-cell signalling

Françoise Pagès, Marguerite Ragueneau, Robert Rottapel, Alemseged Truneh, Jacques Nunes, Jean Imbert & Daniel Olive

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