Binding of IL-4 to the IL-13Rα1/IL-4Rα receptor complex leads to STAT3 phosphorylation but not to its nuclear translocation

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Abstract Interleukin-4 (IL-4) is a pleiotropic cytokine, which acts on both hematopoietic and non-hematopoietic cells, through different types of receptor complexes. In this study, we report that in human B cells, IL-4 caused rapid phosphorylation of Janus kinase (JAK) 1 and JAK3 tyrosine kinases. In keratinocytes, the hematopoietic-specific receptor common γ_c chain is not expressed and the IL-13 receptor α_1 (IL-13Rα_1) participates in IL-4 signal transduction. In keratinocytes, IL-4 induced JAK1 and JAK2 phosphorylation but, unlike in immune cells, IL-4 did not involve JAK3 activation for its signaling. In both cell types, IL-4 induced phosphorylation and DNA binding activation of the signal transducer and activator of transcription (STAT) 6 protein. Furthermore, IL-4 stimulation of keratinocytes also induced tyrosine phosphorylation of STAT3 which was found to bind to the phosphorylated IL-13Rα_1. STAT3 however did not significantly translocate to the nucleus, nor did it bind with high affinity to target DNA sequences.

Key words: Nuclear localization; Signal transducer and activator of transcription; Janus kinase; Signal transduction; Cytokine receptor; Keratinocyte

1. Introduction

Interleukin-4 (IL-4) is a pleiotropic cytokine, which evokes a high variety of biological responses by binding to a high affinity receptor complex. IL-4 receptors (IL-4R) are expressed in low numbers on a wide range of cell types, including T and B lymphocytes, monocytes, granulocytes, fibroblasts, epithelial and endothelial cells. Cross-linking studies have shown that IL-4 could bind to three molecular species of 140 kDa, 70–75 kDa and 65 kDa, indicating a multimeric structure of IL-4R [1]. The 140 kDa IL-4 binding protein (IL-4Rα) is expressed ubiquitously. The 65 kDa protein represents the IL-2Rγc chain, a common component of other cytokine receptors [2–5], which increases by 2–3-fold the affinity of IL-4 for its receptor in lymphoid cells [6] and participates in some of the IL-4-mediated events [2]. The lack of γ_c chain expression on human renal carcinoma cells, which nevertheless efficiently bind IL-4 [7] and respond to IL-4 [8], suggests the participation of another subunit in the IL-4R. In cells lacking γ_c, IL-4 signal transduction is thought to mobilize either homodimerization of IL-4Rα or heterodimerization of IL-4Rα with either one or two IL-13R chains [4,9,10]. Two IL-13Rα cDNAs have recently been cloned and both encode glycoproteins of 70–75 kDa [11–13].

These receptor subunits do not contain any consensus sequences encoding tyrosine or serine/threonine kinases. Engagement of IL-4 with its receptor triggers the Janus family kinases (JAK). Although the phosphorylation level of individual JAK depends upon the cell type studied, tyrosine phosphorylation of all the JAKs (JAK1, JAK2, JAK3, TYK2) has been reported after IL-4 binding [1]. It has been shown that the IL-4Rα associates with JAK1 [14] and the γ_c associates with JAK3 [15,16]. In response to IL-4, tyrosine phosphorylation of members of the signal transducer and activator of transcription (STAT) family has been described. STAT6 activation appears to be a common feature of IL-4 signaling, regardless of the cellular system studied [17].

We report here that the composition of IL-4R differs between keratinocytes and lymphocytes. In lymphocytes, IL-4 signal transduction occurs through an IL-4Rα/γ_c complex. In keratinocytes, in which γ_c is not expressed, our results indicate that IL-13Rα_1 participates to the IL-4R complex. These differences in the receptor composition lead to distinct proximal events in IL-4 signaling. Thus, treatment of lymphocytes with IL-4 resulted in tyrosine phosphorylation of JAK1 and JAK3, whereas JAK1 and JAK2 were activated in keratinocytes. In addition, there was a rapid induction of DNA binding protein complexes containing STAT6 which was tyrosine-phosphorylated in the two cell lines. Interestingly, in keratinocytes, STAT3 underwent partial activation in that it appears to be tyrosine-phosphorylated but does not translocate significantly to the nucleus.

2. Materials and methods

2.1. Cell culture

Keratinocytic cell lines (A431 and SVK14) were cultured in DMEM supplemented with antibiotics (50 μg/ml penicillin, 50 μg/ml streptomycin), with 1 mM sodium pyruvate and with 10% fetal calf serum.

Hematopoietic cell lines (Ramos B lymphocytes, Kit 225 T lymphocytes) were cultured in RPMI supplemented with antibiotics (50 μg/ml penicillin, 50 μg/ml streptomycin), and with 10% fetal calf serum.

2.2. Antibodies and reagents

Human recombinant IL-4, a generous gift of Dr. N. Vita (Sanofi,

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Abbreviations: EGF, epidermal growth factor; EMSA, electrophoretic mobility shift assay; GAS, γ-interferon-activated sequence; GSH, glutathione; GST, glutathione S-transferase; IL, interleukin; IL-4R, IL-4 receptor; IL-13R, IL-13 receptor; JAK, Janus kinase; PAGE, polyacrylamide gel electrophoresis; RT-PCR, reverse transcriptase polynucleotide chain reaction; SH2, Src homology 2; SIE, *sis*-inducible element; STAT, signal transducer and activator of transcription

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France), was added to the culture media at a final concentration of 50 ng/ml. Epidermal growth factor (EGF) was purchased from Sigma and used at a final concentration of 100 ng/ml.

Anti-JAK1 polyclonal antibodies (06-272), anti-JAK2 polyclonal antibodies (06-255) and anti-P-Tyr monoclonal antibodies were from UBI. Anti-STAT3 (C-20), anti-STAT6 (S-20) and anti-JAK3 (C-21) polyclonal antibodies were from Santa Cruz.

2.3. Reverse transcriptase polynucleotide chain reaction (RT-PCR)

To analyze expression of the various IL-4R chains, total RNA was extracted with Trizol (Life technologies) as described by the manufacturer. Primer pairs specific for the different component were: 5′-GCGTCTCCGACTACATGAGC (sense) and 5′-GGTTGCTCCAGGTCAGCAGC (antisense) for IL-4Rα, 5′-CAGCCTACCAACCTCACTCT (sense) and 5′-GTCCTGGAGCTGAACAACAA (antisense) for γ_c, 5′-AGGATGACAAACTCTGGAG (sense) and 5′-CTCAAGGTCACAGTGAAGG (antisense) for IL-13Rα_1, 5′-ATACCTTTGGGACTTATTCC (sense) and 5′-TGAACATTTGGCCATGACTG (antisense) for IL-13Rα_2.

2.4. Immunoprecipitation

Cells were lysed in a RIPA buffer containing 50 mM Tris–HCl, pH 7.5, 1% Triton X-100, 50 mM NaCl, 50 mM NaF, 10 mM NaPP, 5 mM EDTA, 1 mM Na_3VO_4, 1 mM PMSF, 10 μg/ml aprotinin, 10 μg/ml leupeptin, 10 μg/ml pepstatin. The cell lysates were clarified by centrifugation. Five-hundred μg of total protein was incubated with appropriate antibodies. The immunoprecipitates were recovered with protein A-Sepharose beads at 4°C for 1 h, washed, resolved on sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to a Hybond-C extra membrane (Amersham). The blots were then hybridized with the appropriate antibodies.

2.5. Affinity purification of DNA binding proteins

Nuclear extracts were prepared as described for electrophoretic mobility shift assay (EMSA). The oligonucleotide sequences used were derived from the high affinity *sis*-inducible element (SIE) of the *c-fos* gene (SIE m67), sense: 5′-CATTTCCCGTAAATC [18] and from γ-interferon-activated sequence (GAS), sense: 5′-GTATTTCCCAGAAAAGGAAC [19]. DNA binding proteins were isolated from 1 mg of nuclear extracts by incubation at 4°C for 1 h with 3 μg double-stranded 5′-biotinylated oligonucleotide coupled to 100 μl of a 50% suspension of streptavidin-agarose (Sigma). Complexes were washed in binding buffer and eluted by boiling in reducing sample buffer. Bound proteins were separated on 10% SDS-PAGE and Western blotting was performed as previously described.

2.6. Glutathione S-transferase (GST) fusion proteins and GST pull down experiments

A GST-IL-13Rα_1 intracytoplasmic domain fusion protein was constructed as follows. A plasmid encoding IL-13Rα_1 was kindly provided by Dr. N. Vita (Sanofi, France) and was used as a template to amplify the intracytoplasmic domain that was then cloned in a *Bam*HI-*Eco*RI-linearized PGEX-2T. For this purpose, we used the following pair of primers: 5′-ATGCGGATCCGAGGATGACAAACTC (sense) and 5′-GCATGAATTCCTGAGAGGCTTTCTT (antisense). GST fusion proteins were produced either in BL21 or in BL21 expressing the tyrosine kinase Elk and immobilized on glutathione (GSH)-Sepharose beads. 2.5 mg of cellular extracts prepared in buffer used for immunoprecipitation was rocked with coupled beads overnight at 4°C. The beads were washed twice in lysis buffer, boiled in sample buffer and bound proteins were analyzed by Western blotting.

2.7. Immunofluorescence studies

Cell monolayers grown on coverslips were rinsed twice in phosphate-buffered saline (PBS) and fixed in 2% formaldehyde/PBS. After two additional rinses, cells were permeabilized with 1% Triton X-100 (in PBS) and then washed in 0.1% Tween 20. Non-specific protein adsorption was blocked by a 30 min incubation with 2% bovine serum albumin (BSA)/PBS. Samples were then incubated with primary antibodies (1:200 in 2% BSA/PBS) for 1 h (22°C), washed and incubated with secondary antibodies (FITC-conjugated goat anti-rabbit antibodies, 1:300, Nordic) for 30 min. These samples were washed and examined at a magnification of ×40 under a Zeiss LSM410 confocal microscope.

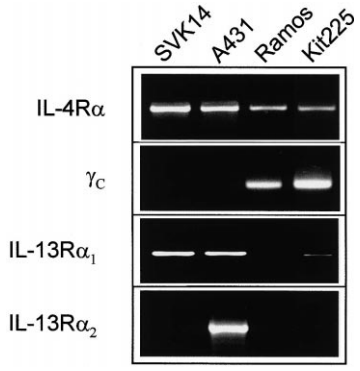

Fig. 1. Composition of the receptor for IL-4. Amplification of the IL-4Rα, γ_c, IL-13Rα_1, IL-13Rα_2 mRNA was performed on total RNA of the different cell lines. The sequences of the oligonucleotides used to amplify the various chains and expected sizes of the PCR products are indicated in Section 2.

3. Results

3.1. Expression of IL-4R components in keratinocyte and in lymphoid cell lines

IL-4 is a pleiotropic cytokine which acts on hematopoietic and non-hematopoietic cells, with different biological activities. Different receptor chains, IL-4Rα, γ_c, IL-13Rα_1 and IL-13Rα_2 have been described to participate in the composition of multiple forms of IL-4R. We therefore analyzed the expression of the different chains by RT-PCR in two human keratinocytic cell lines (A431, SVK14) and in two human lymphoid cell lines, Ramos (B lymphocytes) and Kit 225 (T lymphocytes). The results presented in Fig. 1 indicate that whereas IL-4Rα was present in all cell lines tested, this is not the case for the other putative components of IL-4R. As expected, expression of the hematopoietic-specific γ_c chain was restricted to lymphocytes, and no expression of this chain was observed in keratinocytes. Expression of the IL-13Rα_1 chain was observed in keratinocytes whereas no expression of this chain was detectable in B lymphocytes. The presence of IL-13Rα_2 was only observed in A431 cell lines. These results suggested that IL-4 signaling in keratinocytes might occur through either IL-4Rα homodimerization or heterodimerization with one (SVK14) or two chains (A431) of IL-13R and in lymphocytes through heterodimerization of IL-4R and γ_c.

3.2. Activation of the JAK/STAT pathway in Ramos and in A431 cells

Engagement of IL-4 with its receptor induced triggering of the JAK pathway. We therefore examined the phosphorylation of the different JAKs in the two cell lines in which IL-4 signal transduction could be mediated either by IL-4R/γ_c (Ramos) or by IL-4R/IL-13Rα (A431). Both cell lines were stimulated by IL-4 and total cellular extracts were immunoprecipitated with specific JAK antibodies. Phosphorylation of these proteins was monitored by anti-P-Tyr Western blotting. As shown in Fig. 2A, in the two cell lines, tyrosine phosphorylation of JAK1 was observed after 10 min of IL-4 stimulation.

We then investigated whether JAK2 and/or JAK3 were activated in both cell lines. Following cell stimulation with IL-4, the anti-JAK2 antiserum immunoprecipitated a 130 kDa phosphorylated protein from A431 cell extracts, whereas a

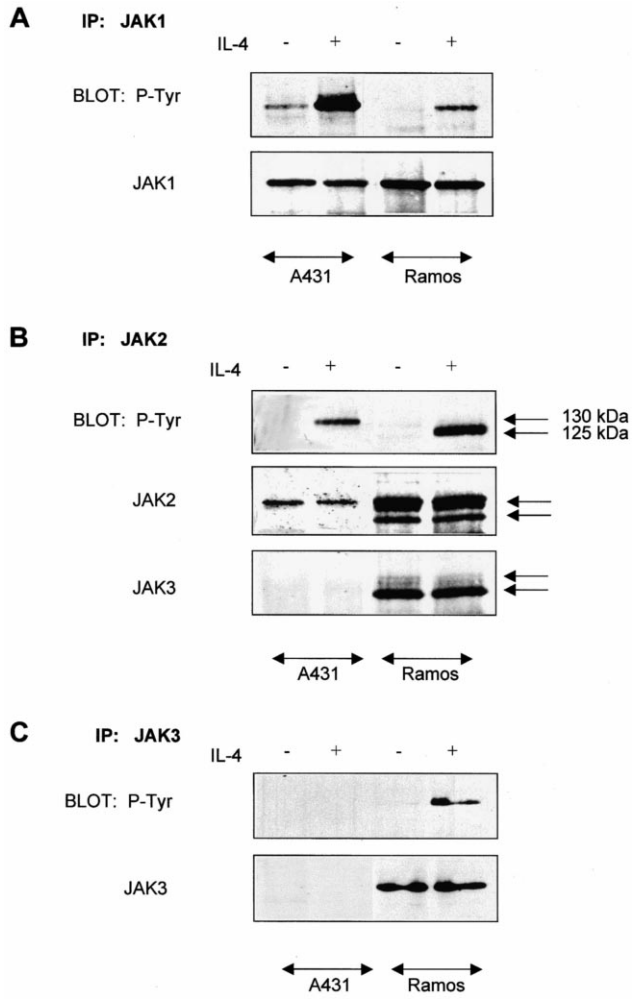

Fig. 2. JAK phosphorylation in response to IL-4 treatment. Ramos or serum-starved A431 cells were stimulated with IL-4 for 10 min (+) or not (−). Cell extracts were immunoprecipitated (IP) with anti-JAK antibodies. A: With anti-JAK1 antibodies followed by Western blotting with anti-P-Tyr antibodies (upper). The blot was then stripped and reprobed with anti-JAK1 antibodies (lower). B: With anti-JAK2 antibodies followed by Western blotting with either anti-P-Tyr antibodies (upper). Blots were then stripped and reprobed with either anti-JAK2 antibodies (medium) or anti-JAK3 antibodies (lower). Molecular weights of tyrosine-phosphorylated protein are indicated. C: With anti-JAK3 antibodies followed by Western blotting with anti-P-Tyr antibodies (upper). Blots were then stripped and reprobed with anti-JAK3 antibodies (lower).

125 kDa phospho-protein was precipitated from Ramos cells (Fig. 2B, upper). It has already been reported that many anti-JAK2 antibodies cross-react with JAK3 proteins [20]. Thus, reprobing the blot with anti-JAK2 antibodies clearly indicated that the 130 kDa protein in A431 corresponded to JAK2 and was not reactive with the anti-JAK3 antiserum (Fig. 2B, medium panel). In contrast, JAK2 was detectable in B cells, but was not tyrosine-phosphorylated. Western blotting with an anti-JAK3 indicated that the phosphorylated protein migrating at 125 kDa was JAK3 (Fig. 2B, lower panel). These results were further substantiated by JAK3 immunoprecipitation that confirmed that upon IL-4 stimulation, JAK3 became tyrosine-phosphorylated in Ramos cells and that it is not expressed in A431 (Fig. 2C). Treatment of the two cell lines with IL-4 failed to induce tyrosine phosphorylation of TYK2 (data not shown).

3.3. Phosphorylation of STATs

The current model of cytokine signaling proposes that upon cytokine binding to its specific receptor, JAKs phosphorylate the receptor on tyrosine residues, forming a docking site for STATs that then become phosphorylated by JAKs. IL-4 has been shown to primarily induce the phosphorylation and activation of STAT6 [17]. In some cellular model however, activation of other STATs has also been described. We therefore investigated the phosphorylation of STATs in these two cell lines by immunoprecipitation of cellular extracts from unstimulated or IL-4-stimulated cells with specific antibodies. We observed a STAT6 phosphorylation (Fig. 3A) in the immunoprecipitates from IL-4-stimulated cellular extracts of the two cell lines. Interestingly, although some constitutive STAT3 tyrosine phosphorylation is observed, IL-4 clearly increased the tyrosine phosphorylation of STAT3 in A431 cellular extracts (Fig. 3B). We have observed no phosphorylation of STAT3 in Ramos (Fig. 3B) nor tyrosine phosphorylation of the other STAT proteins in the two cell lines (data not shown). Taken together, these observations strongly suggested that the presence of IL-13Rα_1 in the IL-4R complex expressed in A431 allowed for JAK2 activation and STAT3 phosphorylation.

3.4. STAT3 interacts with phosphorylated IL-13Rα1 chain

The Src homology 2 (SH2) domain of STAT factors is believed to mediate both binding to the receptor at phosphotyrosine sites and dimerization with a second phosphorylated STAT molecule [21]. Recruitment of STAT6 by IL-4 has been

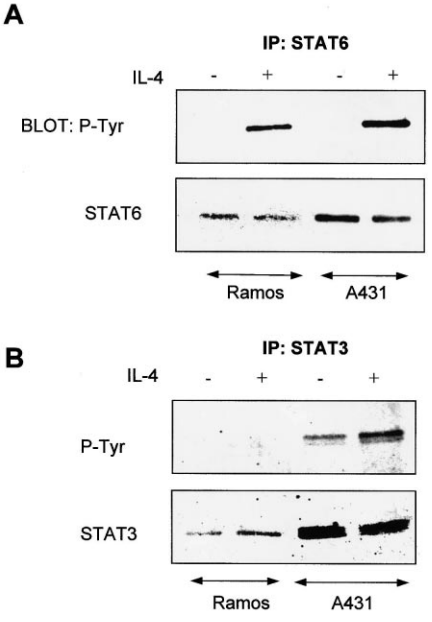

Fig. 3. Phosphorylation of STAT3 and STAT6 in response to IL-4 treatment. Ramos and A431 cells were stimulated (+) or not (−) with IL-4 for 10 min. Cell lysates were prepared and 500 μg of total protein was immunoprecipitated (IP) with either STAT6 (A) or STAT3 (B) antibodies. After separation by SDS-PAGE and transfer to nitrocellulose filter, precipitated proteins were probed with anti-phosphotyrosine antibodies (upper) and with the immunoprecipitating antibodies (lower).

shown to depend on three consensus YK/QXF motifs in the IL-4Rα chain [22], and we hypothesized that the unique participation of IL-13Rα$_1$ in the A431 IL-4R complex might be responsible for STAT3 activation. In many cytokine receptors, recruitment of STAT3 occurs through SH2 recognition of a phosphotyrosine residue in the YXXQ consensus motif [23]. A similar motif YEKQ is present at position 405 in the IL-13Rα$_1$ sequence. We therefore examined whether a GST-IL-13Rα$_1$, containing this consensus motif, could bind STAT3 in vitro. For this purpose, we constructed a GST-IL-13Rα$_1$ fusion protein and produced it either in a regular *Escherichia coli* or in an *E. coli* strain that had been co-transformed with the Elk tyrosine kinase, resulting in GST-IL-13Rα$_1$ being phosphorylated on tyrosine residues. As shown in Fig. 4, neither GST alone nor the non-phosphorylated GST-IL-13Rα$_1$ fusion proteins were able to precipitate STAT3 from A431 cell lysates. When the GST-IL-13Rα$_1$ was used as a tyrosine-phosphorylated protein, a clearcut binding of STAT3 was observed (lane 2, compared to lane 1). This association was abolished by addition of phenyl phosphate, indicating that this protein-protein interaction occurred through a phosphotyrosine residue of IL-13Rα$_1$ with the SH2 domain of STAT3. Under the same conditions, STAT6 did not associate with GST-IL-13Rα$_1$ whether phosphorylated or not (data not shown).

3.5. Function and subcellular distribution of STAT3

STAT proteins are activated by tyrosine phosphorylation which is required for dimer formation, nuclear translocation, DNA binding and transcriptional activity in cells treated with cytokines. The induction of DNA binding activity of STAT3 in the nuclei of IL-4-stimulated A431 cells was investigated, using an m67 SIE oligonucleotide, derived from the SIE of the *c-fos* promoter, which has been described to be a target sequence for STAT3 [24]. In this experiment, a biotinylated SIE oligonucleotide coupled to streptavidin-agarose was used to affinity purify STAT3 from nuclear extracts of IL-4- or EGF-stimulated A431 cells. Proteins were resolved by SDS-PAGE and detected by Western blotting with anti-STAT3 antibodies. STAT3 protein was only detected in nuclear extracts from EGF-stimulated cells (Fig. 5A, a). This result suggests that STAT3 is not present in the nucleus after IL-4 stimulation. If we used a biotinylated GAS oligonucleotide instead of biotinylated SIE, and Western-blotted with anti-STAT6 antibodies, we observed the presence of STAT6 in nuclear extracts from IL-4-stimulated cells but not in EGF-stimulated cells (Fig. 5A, b). The same results were obtained by EMSA analysis (data not shown). Taken together, these observations indicate that, although STAT3 is tyrosine-phosphorylated after IL-4 stimulation, no DNA binding activity of this transcription factor could be detected.

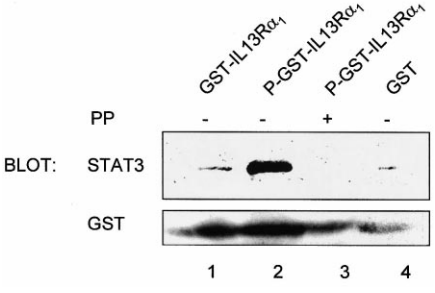

Fig. 4. Association of STAT3 with phosphorylated GST-IL-13Rα$_1$ fusion protein. Cellular extracts from serum-starved A431 were mixed with GST-IL-13Rα$_1$ (lane 1), tyrosine-phosphorylated GST-IL-13Rα$_1$, noted P-GST-IL-13Rα$_1$ (lanes 2 and 3) or with GST alone (lane 4) immobilized on GSH-Sepharose beads. In lane 3, phenyl phosphate (PP) was added to the cell lysates at a concentration of 100 mM. After separation on SDS-PAGE and transfer, proteins were blotted with an anti-STAT3 and anti-GST antibodies.

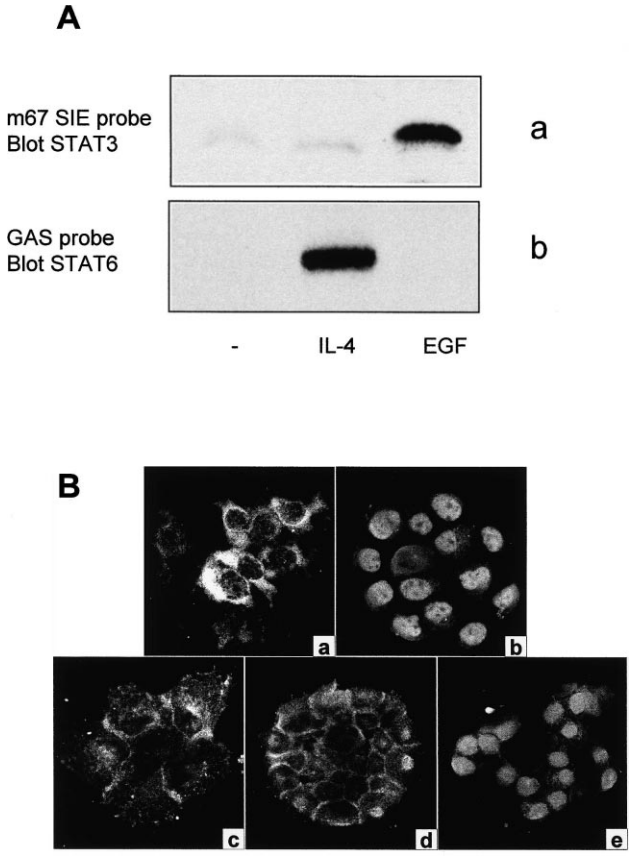

Fig. 5. IL-4 did not lead to a functional STAT3. A: STAT3 DNA binding activity: serum-starved A431 were stimulated with IL-4 or EGF for 10 min or not (−). DNA binding proteins were affinity-purified from nuclear extracts with an agarose-coupled biotinylated m67 SIE (a) or GAS (b) oligonucleotides. Bound proteins were separated by SDS-PAGE and detected with an anti-STAT3 (a) or anti-STAT6 antibodies (b). B: Subcellular distribution of STATs: a and b show STAT6 immunodetection in A431 cells in the absence (a) or in the presence of IL-4 (b). c–e show immunodetection of STAT3 with no stimulation (c), or after IL-4 (d), or EGF (e) stimulation.

In vitro DNA binding of transcription factors does not always reflect their in vivo transcriptional activity. To assess the transcriptional activation of STAT3 by IL-4 and EGF, luciferase-based reporter construct containing a tetramer of the acute-phase response element (APRE)/STAT3 upstream a SV40 minimal promoter was used [25]. When this construct was transfected into A431 cells, induction of the APRE/STAT3 driven luciferase activity was only observed upon EGF stimulation (10-fold). There was no modification of luciferase activity after IL-4 stimulation (data not shown). These results are in agreement with the absence of DNA binding activity of STAT3.

Since neither DNA binding nor transcriptional activities of STAT3 could be detected, we investigated the cellular localization of STAT3 by immunofluorescence assay, in compar-

ison with STAT6. In unstimulated cells, both STAT3 and STAT6 resided predominantly in the cytoplasm (Fig. 5B, a and c). When cells were stimulated with IL-4, STAT6 translocated quickly and massively to the nucleus (Fig. 5B, b). In contrast, after IL-4 stimulation, STAT3 remained largely in the cytoplasm, and it was only clearly detectable in the nucleus of very few cells (Fig. 5B, d). In these experiments, EGF served as a positive control and was found to induce the nuclear translocation of STAT3 in almost every single cell observed (Fig. 5B, e).

Altogether, these results showed that, although STAT3 is tyrosine-phosphorylated, its nuclear translocation, and therefore its DNA binding and transcriptional activity, are not activated by IL-4.

4. Discussion

Within the last few years, studies on the structure and signaling capacity of the IL-4R have elucidated mechanisms whereby IL-4 regulates its panoply of biological responses. While the structure of IL-4R has been extensively studied, it has not been fully characterized especially in non-immune cells. Our data demonstrate that the IL-4R complex in human keratinocytes is different from that expressed in lymphocytes. Unlike lymphocytes, keratinocytes do not express the hematopoietic cytokine receptor common γ_c. Characterization of IL-4R, reported in a larger number of cell lines, indicated that only hematopoietic cells significantly expressed γ_c, whereas there was no expression of this chain in any of the non-hematopoietic tumor cell lines examined [26]. Different chains, able to participate to the formation of the IL-4R, are expressed in keratinocytes. They are IL-4Rα, IL-13Rα_1 and IL-13Rα_2. Certain discrepancies concerning the expression of IL-13Rα_2 in A431 cells have been reported. Whereas Caput et al. described a low expression of the IL-13Rα_2 chain, Murata et al. reported no presence of this chain in the same cell line [13,26]. Therefore, in keratinocytes, IL-4 is thought to mobilize heterodimerization of one chain of IL-4R with either one or two IL-13R chains. IL-13Rα_1 by itself does not bind IL-4, but when expressed with IL-4Rα, in transfected cells, can induce high affinity binding to IL-4. Co-transfection of IL-4R with IL-13Rα_2 neither modulated IL-4 binding activity nor allowed for STAT6 activation in response to IL-4, indicating that IL-13Rα_2 seems dispensable for IL-4 signal transduction [27]. Therefore, the different putative composition of the IL-4R complexes strongly suggested that IL-4 signaling pathways would be different according to the nature of the receptor. Indeed, in keratinocytes (A431 cells), we have previously reported that the extracellular-regulated kinase pathway was activated in response to IL-4 [28] in contrast to lymphocytes [29].

In the present study, we have focused our investigation on the JAK/STAT pathway in A431 and Ramos cell lines. In Ramos, IL-4 stimulation leads to JAK1, JAK3 and STAT6 phosphorylation, as previously described in other hematopoietic cell lines [30–33]. In A431 cells, our data demonstrate that IL-4 stimulation induces the activation of JAK1 and JAK2 tyrosine kinases. In these cells, no expression of JAK3 was observed. Previous results indicate that in contrast to the other member of the JAK family, expression of JAK3 is mainly observed in cells of lymphoid and myeloid lineages, such as NK cells, T lymphocytes and activated monocytes [32,34,35]]. Further studies have shown that a low level of JAK3 could be observed in endothelial cells [36] or abnormally expressed in transformed hematopoietic and epithelial cell lines such as breast cancer cells [37,38].

In non-hematopoietic cells that lack γ_c, it has been shown that JAK1, JAK2 and TYK2 can be phosphorylated and activated in response to IL-4 [8]. More recently, transfection of CHO cells with the different putative components of IL-4R, IL-4Rα and/or IL-13Rα indicates that the presence of IL-4R and IL-13Rα_1 is required for JAK1 and JAK2 activation leading to an optimal activation of STAT6 in response to IL-4 [27].

The region of the IL-4Rα chain between amino acids 557 and 667 has been shown to play an important role in activation of STAT6 after IL-4 stimulation, as it contains three STAT6 SH2 binding consensus sequences YK/QXF [22]. A STAT3 consensus motif YXXQ has been described in the gp130 family [23]. A similar motif YEKQ is present in the IL-13Rα_1, suggesting that this receptor chain could interact with STAT3. Experiments with GST-IL-13Rα_1 fusion protein tyrosine-phosphorylated in vitro clearly indicated that there is an interaction between STAT3 and IL-13Rα_1. This interaction is probably mediated through the SH2 domain of STAT3 with the phosphorylated tyrosine residue in the YpEKQ motif in IL-13Rα_1, since it was disrupted in the presence of phenylphosphate.

In addition to phosphorylation and activation of STAT6, phosphorylation of STAT3 after IL-4 stimulation has been reported in normal tonsil B cells [39]; whether IL-13Rα_1 is responsible for this activation has not been investigated.

Although STAT3 is tyrosine-phosphorylated after IL-4 stimulation, we observed no DNA binding activity in nuclear extracts nor transcriptional activity of this transcription factor. Furthermore, immunofluorescence studies reported here showed that although STAT3 translocation in response to EGF is normal, IL-4 did not induce significant translocation of STAT3 to the nucleus. The active nuclear import of a large number of nuclear proteins, including STATs, occurs through the Ran/importin pathway [40]. Molecules utilizing this nuclear import system require specific nuclear localization sequences (NLS). Paradoxically, such NLS are not identifiable on STAT proteins [41]. The complete molecular events leading to the translocation of STAT proteins are not fully understood. Recent studies have reported that STAT tyrosine phosphorylation is not sufficient to allow its nuclear translocation. STATs have been shown to interact with cellular components which can be mitogen-activated protein kinase [42], GR [43], PI3 kinase [44] and a number of nuclear transcription factors [45], which could participate in the migration of STAT to the nucleus. Moreover, the involvement of the chaperone GRP58 protein in regulating the transit of phosphorylated STAT3 from the cell membrane to the nucleus has been suggested [46]. GRP58 has been described to be tyrosine-phosphorylated by the Lyn kinase, suggesting the participation of extracellular stimuli in this regulation [47]. Other possibilities that could explain that STAT3 is present in the nucleus at a low level after IL-4 stimulation could be proposed. For example, in particular in the model of prolactin receptor, the number of phosphorylated tyrosines on the receptor itself seems to play a critical role in the activation of a certain cellular component that modulates STAT5 complexes in the cytoplasm, controlling their nuclear entry [48]. Further studies will be required to

determine how the structural organization of receptors and subsequent activation of particular signaling pathways could affect STAT protein nuclear translocation.

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