STAT6 is required for the regulation of IL-4-induced cytoskeletal events in B cells

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Abstract

During lymphocyte activation, changes in cell morphology are commonly observed. This reflects cell functions important for the regulation of immune responses such as cell adhesion or cell migration. Notably, IL-4 has been shown to induce adhesion and locomotion in B cells, and we have recently described that IL-4 causes dramatic changes in B cell morphology. Thus, such B cells spread with dendritic cell protrusions and produce microvilli-like structures. The molecular mechanisms by which IL-4 induces these complex changes are currently unknown. Two signal transduction pathways are well described for IL-4, i.e. one involving insulin receptor substrate (IRS)-2 and a Janus kinase (JAK)/signal transducer and activator of transcription (STAT) pathway mediated by STAT6. In this study we therefore used B cells from STAT6-deficient mice to address the question of a possible STAT6 dependence in IL-4-induced morphology changes. By light and electron microscopy, cell spreading and polarization were found to be severely impaired and microvilli formation was reduced. In contrast, only mild impairment was observed in cell adhesion in B cells from STAT6-deficient mice. Our results show that adhesion can be induced in the absence of STAT6. However, expression of STAT6 is necessary for optimal responses in both cell adhesion and microvilli induction. STAT6 is also essential to allow an IL-4-dependent spreading or polarization response. A possible interpretation of our results is that STAT6-dependent expression of a specific gene or genes is required for IL-4 to affect changes in B cell morphology.

Introduction

IL-4 influences immune responses by initiating a variety of biological activities affecting different cell types (1). The diversity in cellular responses is reflected at the level of signaling and two signal transduction pathways for IL-4 are well described (2). In common with other cytokines, IL-4 regulates transcription of specific genes by activating a signal transducer and activator of transcription (STAT) protein, i.e. STAT6 (3). Upon occupation of the IL-4 receptor (IL-4R), STAT6 proteins are tyrosine phosphorylated, and dimerize at the cell surface and subsequently translocate to the nucleus where they initiate gene transcription (4). In a second pathway, occupation of the IL-4R leads to tyrosine phosphorylation of insulin receptor substrate (IRS)-2 (previously referred to as 4PS) (5). Phosphorylated IRS-2 in turn recruits other molecules active in signal transduction to elicit further downstream effects. Such molecules include the p85 subunit of phosphatidylinositol 3-kinase (PI3-kinase), the adapter protein Grb2 and the protein tyrosine phosphatase PTP2 (6).

Perhaps the most profound effects induced by IL-4 are found in B lymphocytes. IL-4 induces Ig class switching to IgE and IgG1 (mouse) or IgG4 (human), and germline transcription of their respective heavy chain genes (ε and γ1 or γ4) has been shown to precede and be necessary for gene recombination (for review, see 7). The transcriptional activation of γ1 and ε appears to involve STAT6 promoter binding (8–10). Work using gene-targeted mice shows that STAT6 is essential to allow IL-4 induced activation of IgE and optimal IgG1 responses (11,12). Also, IL-4 increases expression of MHC class II and CD23 in a STAT6-dependent manner (11,14).

A number of reports provide evidence for IL-4 regulating changes in the cytoskeleton and influencing cell morphology. Cell motility was induced by IL-4 in human (15) and mouse B cells (16). Here cells were seen to polarize to an elongated morphology (17). Enhanced B cell aggregation was induced by lipopolysaccharide (LPS) plus IL-4 (mouse) or anti-CD40 plus IL-4 (human) stimulation, resulting in tight cell aggregates (18–20). IL-4 has also been shown to regulate cytoskeleton directly in endothelial cells and neutrophils (21,22). We have recently reported the ability of IL-4 to induce cell spreading in LPS-stimulated B cell blasts. B cells thus stimulated were
found to attach to antibody-coated tissue culture surfaces and spread out, forming long dendrites. Similar cell spreading was observed in anti-CD40-stimulated B cells. Anti-CD40, which mimics the action of CD40 ligand, like IL-4 is a T cell-derived signal and we have suggested that the spreading phenomenon may represent events in cell–cell interactions possibly between B cells and T cells (23). In these studies, we also observed that LPS plus IL-4-activated cells formed microvilli-like surface structures. Our recent studies indicate that cell surface microvilli have a direct role in cell adhesion (submitted for publication). These activities are consistent with IL-4 influencing B cell localization and interaction with other cells, and suggest a broader role for IL-4 in B cell activation. It is currently not clear how IL-4 can regulate the observed complex changes in cell morphology. In this paper we use B cells derived from STAT6−/− mice in order to investigate the role of STAT6 in IL-4-induced morphological changes.

Methods

Reagents and antibodies

Purified recombinant murine IL-4, LPS, anti-CD40 mAb 1C10, anti-IgM mAb Ak13, Sepharose-coupled Ak13, anti-CD44 mAb RK3G9, anti-CD23 mAb B3B4, anti-LFA-1 α-subunit mAb FD448.1 and anti-CD4 mAb L3T4 were purified and used as previously described (23). Rat anti-mIL-4R antibodies (1688-01; R & D Systems, Minneapolis, MN), rat IgG2a (11020D; PharMingen, San Diego, CA) and FITC-labeled mouse anti-rat IgG (212-096-082; Jackson ImmunoResearch, West Grove, PA) were used as detailed below.

Animals

STAT6−/− mice were kindly provided by Dr James Ihle (Memphis, TN) (12), and were bred and maintained in the animal facility at the Department of Cell and Molecular Biology, Karolinska Institutet. STAT6+/−×STAT6+/− breeding was established to provide litters of mixed genotype. The genotype of the mice was controlled by PCR on tail prepared DNA. Most experiments were performed with sibling littermates. In a number of experiments, CBA/J×C57BL/6 F1 mice (purchased from Charles River, Uppsala, Sweden and maintained in the same animal facility) were used as homozygous wild-type controls.

Cell culture

Individual B cell preparations were made from mice with a defined STAT6 genotype. Single-cell suspensions from individual spleens were depleted from T cells by complement treatment in the presence of anti-T cell antibodies and subjected to Percoll centrifugation as previously described (24), with the exception that cells were collected from the interphase of 50 and 70% Percoll. Cells were cultured in RPMI 1640 supplemented with 2 mM L-glutamine, 1 mM sodium pyruvate, 50 IU/ml penicillin, 50 µg/ml streptomycin, 50 µM 2-mercaptoethanol and 5–10% heat-inactivated FCS at 37°C in a humidified atmosphere containing 5% CO2.

DNA synthesis assays

B cells prepared from STAT6−/−, STAT6+/− and STAT6+/+ mice were resuspended to a concentration of 5×10^5/ml, distributed at 200 µl/well in 96-well plates and stimulated as indicated. After 48 h [3H]thymidine was added to a final activity of 2 µCi/ml and the cells were cultured for a further 16 h prior to harvesting and measurement of activity using a β-counter.

Cell spreading assays

Cell spreading assays were performed in antibody-coated 96-well plates as previously described (23). Briefly, B cells were stimulated as indicated in the figure legends, transferred to antibody-coated plates and incubated for various times at 37°C. The cells were then fixed with glutaraldehyde and the percentage of spread cells (here defined as having at least one dendritic process of at least one cell diameter) was determined.

FACS analysis

The surface expression of CD44, IgM, LFA-1, CD23 and CD4 (control) on LPS plus IL-4-stimulated blasts from STAT6−/− and STAT6+/+ mice was determined as follows. Cells stimulated for 3 days were stained with primary antibody (20 µg/ml) in PBS containing 1% BSA and 2% mouse serum followed by FITC–F(ab′)2 mouse anti-rat IgG (Jackson ImmunoResearch) at a dilution of 1/50. FACS analysis was performed on a FACScan II machine (Becton Dickinson, San Jose, CA) using CellQuest software.

Expression of IL-4R was determined by first incubating 2×10^6 cells with 50 µl mouse serum for 30 min, followed by incubation with anti-IL 4R antibodies (20 µg/ml, 100 µl) or control IgG2a antibodies in 50% mouse serum for 1 h on ice. Thereafter cells were washed in the cold, resuspended in FITC-labeled mouse anti-rat IgG diluted 1/40, incubated for 30 min on ice and analyzed in a FACS Calibur (Becton Dickinson). In the analysis, single cells were gated using forward scatter and dead cells were excluded by propidium iodide staining.

Aggregation assay

B cells from STAT6−/− or STAT6+/+ mice were distributed in 96-well plates, at 200 µl/well volumes and stimulated with LPS plus IL-4, LPS or anti-CD40 for 42–46 or 66–70 h, after which cell aggregation was assayed. Trypan blue was added and the cells were then resuspended by gently pipetting up and down 6 times in a blunted disposable pipette tip and transferred to a hemocytometer. Aggregated (two or more cells in association) and single cells were counted under a light microscope and the percentage of aggregated cells calculated. At least 300 cells were counted for each determination.

Cell polarization assays

Cell polarization assays were employed to assess the induction of a motile phenotype in B cells. This phenomenon has previously been shown to correlate with the activation of lymphocyte locomotion (17). Cells from STAT6−/−, STAT6+/− or STAT6+/+ mice were cultured for 20 h in 500 µl volumes at 0.5×10^6 cells/ml in 24-well tissue culture plates in the...
presence of IL-4, anti-CD40, LPS or no stimulus (0.1% BSA in PBS was added as a diluent control for IL-4). The cells were then fixed with glutaraldehyde and the percentage of polarized cells determined using a light microscope as previously described (16). Polarized cells had a tapered body and uropod, whereas non-polarized cells were spherical. The polarized cells described in this assay could be distinguished from spread cells described earlier, based on the fact that they did not possess long dendrites and readily detached from the tissue culture plastic.

**Electron and light microscopy**

For electron microscopy, cells were fixed in 3% cacodylate-buffered (pH 7.3) glutaraldehyde, post-fixed in 2% cacodylate-buffered OsO_4, dehydrated in graded ethanol, stained with 2% uranyl acetate in ethanol and embedded in Spurr a low viscosity epoxy resin as previously described (23). Thin sections were cut with an LKB Ultrotome IV, stained with lead citrate and examined in a Philips CM120TWIN electron microscope.

For light microscopy, cells were fixed (2.5 % glutaraldehyde) in tissue culture plates, and then examined and photographed in an inverted microscope using a ×20 objective.

**Results**

**DNA synthesis assays**

We initially used DNA synthesis assays to control that B cells from STAT6^{+/−} mice were capable of responding to IL-4. Anti-Ig and IL-4 synergize to produce a strong, proliferative response in primary B cells (25). It has been reported that STAT6^{+/−} mice were capable of responding to IL-4 in combination with anti-Ig, albeit at diminished levels (12). On the other hand, Takeda et al. reported no response in their gene-targeted mice (11). In Fig. 1 we show that IL-4 clearly synergized with anti-IgM-coupled Sepharose in inducing proliferation, although to a lower level than seen in cells from STAT6^{+/+} and STAT6^{+/−} mice. A lower response in STAT6-deficient B cells was observed both at suboptimal and saturating doses of IL-4 (data not shown). STAT6^{−/−} cells showed a strong proliferative response to LPS and anti-CD40 stimulation, comparable to that of the STAT6^{+/−} and STAT6^{+/+} cells, indicating that there was no intrinsic defect in proliferation in the absence of STAT6 (Fig. 1).

The IL-4 receptor is expressed in the absence of STAT6

IL-4 has previously been shown to induce expression of its own receptor (26,27). We therefore examined expression levels of IL-4R in STAT6-deficient B cells using FACS analysis. Non-stimulated B cells from wild-type or STAT6^{+/−} mice expressed low but detectable levels of IL-4R (Table 1). One hour after stimulation with IL-4, the receptors were completely down-regulated, most likely due to ligand-induced internalization. After 24 or 48 h of culture, LPS had induced up-regulation in the amount of IL-4R per cell, in both wild-type and STAT6-deficient B cells. In cells from STAT6^{+/+} mice there was marked increase in IL-4R expression by IL-4 plus LPS and a modest response to IL-4 alone at 24 and 48 h. STAT6-deficient B cells stimulated in the presence of IL-4, with or without LPS, maintained low or undetectable levels of IL-4R at 24 and 48 h. In fact, STAT6^{−/−} cells stimulated with LPS plus IL-4 had a lower level of IL-4R expression than cells induced by LPS alone. A possible explanation of these results is that internalization of the receptor occurred after ligation.
Fig. 2. IL-4-induced B cell spreading is severely impaired in STAT6-deficient animals. B cells from STAT6+/+; STAT6+/-; or STAT6-/- mice were cultured with stimuli as shown for 44 h and transferred to anti-CD44 (RK3G9)-coated 96-well plates. The cells were then cultured for a further 16 h after which they were fixed. Triplicate determinations were counted for cell spreading. Mean values are presented and error bars represent SD (A). The fixed cells were also photographed using an inverted microscope (B). B cells from STAT6+/+ (a, c and e) or STAT6+/- (b, d and f) mice were cultured with LPS alone (a and b), LPS + IL-4 (a and b), LPS alone (c and d) or anti-CD40 (e and f). Bar = 20 µm. The data are representative of three similar experiments.

Fig. 3. IL-4-induced B cell spreading is impaired also after shorter IL-4 exposures. B cells from STAT6+/+ or STAT6-/- mice were activated by LPS (empty and hatched bars) or LPS + IL-4 (black bars) for 2 days and were thereafter re-cultured on anti-CD44-coated plastics for 18 h in the presence of LPS (empty bars) or LPS + IL-4 (hatched and black bars), after which the spreading response was determined. The data are representative of three similar experiments.

IL-4-induced B cell spreading is severely impaired in STAT6-/- mice

B cells stimulated with LPS or anti-Ig in combination with IL-4 (but not IL-4 alone), or anti-CD40 will spread on surfaces coated with antibody recognizing B cell surface determinants. The spread cells stained with the B cell-specific antibody B220, showing that they were indeed B cells (23). LPS plus IL-4-induced spreading of B cells from STAT6-/- animals was shown to be severely impaired in cells cultured on anti-CD44 (RK3G9)-coated surfaces, while a mildly impaired response was seen with STAT6+/- cells (Fig. 2A and B). Occasional cells showing pronounced spreading were seen in LPS plus IL-4-stimulated STAT6+/- cultures, suggesting that the response was not totally abrogated in the absence of STAT6. B cells stimulated with LPS alone failed to spread to a significant degree, while cells treated with anti-CD40 gave a strong spreading response irrespective of STAT6 genotype. This demonstrates that B cells were not intrinsically deficient in their ability to spread in the absence of STAT6. CD40 has recently been reported to induce the binding of STAT6 to promoter constructs (28). Our results further demonstrate that induction of B cell spreading via CD40 is not dependent on STAT6.

We showed above that the IL-4R was down-regulated by IL-4 in STAT6+/- B cells. There is a possibility that the lack of spreading in STAT6+/- B cells was due to absence of IL-4R. This was tested by using shorter incubation times with IL-4. Instead of culturing B cells with IL-4 from the beginning, STAT6+/- or wild-type B cells were cultured with LPS alone for 2 days. Thereafter, cells were placed on antibody-coated surfaces in the presence of IL-4 and incubated for an additional 18 h. However, the spreading response was not
improved in STAT6-deficient B cells (Fig. 3). This would argue that the lack of spreading in STAT−/− B cells is not simply due to lack of IL-4R expression.

IL-4 has previously been shown to regulate the cell surface expression of certain proteins, such as CD23 (10) and MHC class II (14), in a STAT6-dependent manner. We have used anti-CD44 coating of tissue culture wells for the spreading assay in this as well as in a previous study. We demonstrated that spreading could be induced by a variety of antibodies specific for different B cell surface proteins (23). In order to address the possibility that the deficiency in cell spreading observed in STAT6−/− cells (shown above) was due to lack of CD44 expression or specific to CD44, we examined the ability of cells to spread on surfaces coated with various antibodies. We also analyzed the expression levels of the cell surface antigens by FACS. STAT6−/− B cells stimulated with LPS plus IL-4 were found to be deficient in spreading on surfaces coated with antibodies directed to LFA-1, IgM or CD44 (Fig. 4A). FACS analysis performed with the same cells revealed similar expression levels of IgM and LFA-1 in LPS plus IL-4-stimulated B cells from STAT6−/− and STAT6+/+ mice, while CD44 was found to be expressed at approximately twice the level on wild-type cells (Fig. 4B). STAT6−/− cells show markedly lower levels of CD23 expression after LPS plus IL-4 stimulation than that of STAT6+/+ cells (Fig. 4B), as previously shown (11). Thus, the deficiency in spreading in STAT6−/− B cells is not specific for anti-CD44 induction and cannot be explained only by lack of expression of the cell surface antigens.

The microvilli response is reduced in LPS plus IL-4-stimulated STAT6−/− cells

We have previously reported the induction of microvilli-like cell surface protrusions in B cells stimulated by LPS plus IL-4 but not by LPS alone (23). Here, we asked if this response could be supported also in STAT6−/− cells. Electron microscopy studies revealed that villous surface structures and extended dendritic processes were formed in STAT6+/+ cells stimulated with LPS plus IL-4, but not with LPS alone (Fig. 5A and B). With STAT6−/− B cells the difference was smaller and LPS plus IL-4 induced only a few more processes than LPS alone (Fig. 5C and D), and clearly fewer than in similarly activated STAT6+/+ cells (Fig. 5B).

LPS plus IL-4-induced B cell aggregation is mildly impaired in the absence of STAT6

The ability of IL-4 to stimulate higher levels of aggregation in LPS blasts has been previously reported (18, 20). STAT6−/− B cells cultured for 2 days (42–46 h) in the presence of LPS plus IL-4 showed consistently lower levels of cell aggregation as compared to their wild-type counterparts (Fig. 6). On day 3 (66–70 h), however, no difference was seen in the levels of aggregation when comparing STAT6−/− and wild-type B cells thus stimulated.

LPS-stimulated B cell blasts formed large and loose aggregates in culture, whereas cells stimulated with LPS in combination with IL-4 formed large, tight aggregates often adherent to the tissue culture well. Additionally, some spread

![Fig. 4. Impaired IL-4-induced B cell spreading in STAT6-deficient mice](image-url)
cells were seen in cultures exposed to LPS plus IL-4 but not LPS alone, as previously described (18). In contrast, STAT6+/− B cells, induced by LPS plus IL-4, showed almost no spread cells and the cell aggregates failed to adhere strongly to the tissue culture plastic but detached easily by gently rocking (data not shown).

**Fig. 5.** Microvilli induction is impaired in STAT6+/− B cells. Electron micrographs of B cells cultured with LPS (A and C) or LPS plus IL-4 (B and D). Cells from STAT6+/+ mice (A and B) and STAT6+/− mice (C and D) are shown. Note the formation of villous surface structures and extended dendritic processes in cell contacts of LPS plus IL-4-treated wild-type cells (B). Cell contacts between smooth surfaces are also seen (arrows). LPS-stimulated cells (A and C) or LPS plus IL-4-stimulated STAT6+/− cells (D) have smoother surfaces and fewer microvilli. Bars = 2 µm.

**Induction of B cell polarization by IL-4 is impaired in the absence of STAT6**

Lymphocytes undergoing locomotion display an elongated or polarized cell morphology. This has previously been described as the basis for a method to measure induction of a motile phenotype in lymphocytes (17). It was also shown
Fig. 6. LPS plus IL-4-induced B cell aggregation is mildly impaired in the absence of STAT6. B cells from STAT6<sup>−/−</sup> or STAT6<sup>+/+</sup> mice were cultured with indicated stimuli for two or three days, as indicated, after which the percentage of aggregated cells was determined. The data shown are means of triplicates and error bars represent SD. One representative experiment of three is presented.

Fig. 7. Induction of B cell polarization is severely impaired in the absence of STAT6. B cells from STAT6<sup>−/−</sup>, STAT6<sup>+/−</sup> or STAT6<sup>+/+</sup> mice were cultured with stimuli as shown for 20 h after which they were fixed and triplicates counted for cell polarization. Results are shown as means ± SD. The data from IL-4 and anti-CD40 stimulation are representative of at least three experiments, while the LPS data is representative of two experiments.

Discussion

IL-4 has been shown to activate a wide variety of genes via STAT6. The role of STAT6 in the regulation of γ1 and ε promoters involved in class switching to IgG1 and IgE has been extensively studied (8,9,13,30,31). Other genes that are regulated by IL-4 in a STAT6-dependent manner include MHC class II, CD23, IL-4 and IL-4Rα (10,14,27,32,33). Furthermore, Th2 cytokine responses are lacking in STAT6-deficient mice (11,12,34), illustrating the breadth of STAT6-mediated signaling. We have recently reported the ability of IL-4 to induce morphological changes in B lymphocytes (23). Here cells were observed to spread with a dendritic morphology and collagen gels in the presence or absence of IL-4, suggesting that there was no intrinsic defect in the ability of cells to migrate in the absence of STAT6 (not shown).

that IL-4 and LPS when added separately induce polarization in B cells (16). Thus, polarization can be induced by IL-4 alone, whereas spreading is only induced by the combination of LPS plus IL-4. We have tested B cells from STAT6<sup>−/−</sup>, STAT6<sup>+/−</sup> and STAT6<sup>+/+</sup> mice for their ability to polarize in response to IL-4, anti-CD40 and LPS. The latter two factors have earlier been shown to induce cell polarization (16,29). STAT6<sup>−/−</sup> B cells showed no significant response to IL-4 (Fig. 7). Over 25% of cells polarized in STAT6<sup>+/−</sup> and STAT6<sup>+/+</sup> cell cultures, when stimulated with IL-4. Anti-CD40 and LPS gave good responses irrespective of genotype. Furthermore, both STAT6<sup>−/−</sup> and STAT6<sup>+/+</sup> cells were capable of penetrating aggregation.

IL-4 induction of cell polarization (an assay used for measuring cell motility) was much reduced have earlier been shown to induce cell polarization (16,29). STAT6<sup>−/−</sup> B cells showed no significant response to IL-4 (Fig. 7). Over 25% of cells polarized in STAT6<sup>+/−</sup> and STAT6<sup>+/+</sup> cell cultures, when stimulated with IL-4. Anti-CD40 and LPS gave good responses irrespective of genotype. Furthermore, both STAT6<sup>−/−</sup> and STAT6<sup>+/+</sup> cells were capable of penetrating aggregation.
IL-4 up-regulates the expression of its own receptor (26,35). More recently, the IL-4R promoter was shown to contain STAT6 sites and IL-4R expression was regulated in a STAT6-dependent manner (33). We found that the IL-4R was equally expressed in splenic B cells from STAT6-deficient and wild-type mice. Up-regulation in receptor expression by LPS was also similar in the two B cell populations. Interestingly, IL-4 induced a rapid down-regulation of its receptor in both wild-type and STAT6−/− B cells. In wild-type B cells, there was an up-regulation of IL-4R at later times, but this did not occur in STAT6−/− B cells. The rapid down-regulation could be due to ligand-induced internalization. The reason why IL-4 levels stayed low in STAT6−/− cells could be due to a slower re-expression of the receptor in the absence of STAT6. The fact that IL-4 down-regulates its own receptor in the absence of STAT6 most likely aggravates the deficiency of the IL-4 response, at least those responses which show slow kinetics.

While we argue that IL-4R down-regulation is not sufficient to explain the deficiency in cell spreading in STAT6−/− mice (see Results), caution is needed in interpreting impairment of function from STAT6−/− mice.

STAT6 has previously been implicated in the regulation of macrophage morphology (36). Macrophages from STAT6−/− mice failed to spread in response to IL-13 (a cytokine sharing many qualities with IL-4) which indicates that this response was mediated by a STAT6-dependent mechanism. Induction of polarization in B cells appears to correlate with elevated levels of general transcription in individual cells and the induction of migration appears to depend on G0 to G1 transition (29). The involvement of STAT6 in this transition is not known.

It is currently not known which genes are affected by STAT6 in order to regulate B cell morphology. STAT6-deficient B cells are capable of responding normally to CD40 treatment in spreading, adhesion and polarization assays, suggesting that the defect is not at the effector level. While our results indicate a role for STAT6 in regulating B cell adhesion, they clearly do not exclude this being mediated by a STAT6-independent mechanism, e.g. via IRS-2. The insulin receptor is known to mediate signaling via IRS-1 and -2. Insulin stimulation results in heavy tyrosine phosphorylation of IRS-1, resulting in SH2 acceptor sites (6). Many homologues of such sites are found in IRS-2. IRS-1 acts as a docking molecule, by recruiting various proteins via SH2 interactions. Insulin is reported to promote cytoskeletal changes, adhesion and migration, in different cell types (37–39) and there is now some evidence to suggest this can be mediated by IRS-1 (39,40). A possible scenario for how IL-4 induces morphological changes could involve IL-4 induction of IRS-2 tyrosine phosphorylation and a STAT6-regulated IRS-2 binding protein or downstream factor. Another possibility is that STAT6 acts as an adapter and binds an IRS-2-regulated protein such as PI3-kinase, as has been demonstrated for STAT3 (41). A third alternative is that two independent signaling pathways, one involving STAT6 and the other IRS-2, cooperate to induce morphology changes in B cells.

Small GTPases of the Rho family have previously been implicated in the regulation of the cytoskeleton (42–44). We are currently investigating if the Rho family members are involved in alterations of B cell morphology. Our preliminary findings indicate that Cdc42 and Rac induce filopodia and lamellipodia formation respectively in B cells. It will be interesting to investigate a possible link between STAT6 signaling and activation of Rho family members as a result of IL-4 stimulation.

In conclusion, our results suggest that expression of a STAT6-dependent factor or factors is necessary for optimal responses in IL-4-induced regulation of B cell morphology. These findings lead to the possibility of cloning the gene or genes involved in regulation of morphology induced STAT6. Thereafter, we can analyze the role of their gene products for physiological in vivo responses of B cells.

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Abbreviations

IL-4R IL-4 receptor
IRS insulin receptor substrate
JAK Janus kinase
LPS lipopolysaccharide
PI3-kinase phosphatidylinositol 3-kinase
STAT signal transducer and activation of transcription

References


