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Regulation of Cell Morphology in B Lymphocytes by IL-4: Evidence for Induced Cytoskeletal Changes

Edward J. Davey,* Johan Thyberg,* Daniel H. Conrad,† and Eva Severinson2* 

Lymphocyte activation is often accompanied by changes in cell morphology, for example, in cell adhesion or motility. IL-4 is a cytokine exerting many effects on B lymphocytes. In this study, we show that stimulation with LPS in combination with IL-4, but not LPS or IL-4 alone, results in a pronounced dendritic morphology of B cells. Using a culture system in which Abs directed to B cell surface markers are immobilized on the tissue culture plastic, we find that cell spreading can be mediated by a variety of Abs, including anti-CD44, -CD23, -LFA-1, -VLA-4, -ICAM-1, and -Ig. B cells stimulated with anti-Ig Abs plus IL-4, or anti-CD40 Abs in the presence or absence of IL-4, are also induced to spread, while IL-2, IL-5, or IL-10 in combination with LPS or alone fail to induce this. Spreading correlates with induction of tight cell aggregation. It is sensitive to cytochalasin B, indicating a requirement for intact actin cytoskeleton. CD44 is selectively detected in the detergent-insoluble fraction of cell lysates prepared from LPS plus IL-4-stimulated B cell cultures after Ab cross-linking of CD44, suggesting a membrane protein-cytoskeleton interaction. Interestingly, electron microscopy studies reveal induction of microvilli-like structures on LPS plus IL-4-stimulated blasts, suggesting that IL-4 can influence cell morphology on an ultra-structural level. In summary, our data show that stimulation with LPS plus IL-4 or ligation of CD40 is capable of inducing dramatic morphologic changes in murine B cells, which correlates with in vitro induction of strong cell adhesion. The Journal of Immunology, 1998, 160: 5366–5373.

We have focused this investigation on the role of IL-4 in inducing cell spreading of primary B cells. IL-4 is a cytokine exerting many effects on different cell types. Perhaps the most profound effects are found on B lymphocytes, where it induces Ig class switching to IgE and IgG1 (mouse) or IgG4 (human), increased expression of MHC class II and CD23, and proliferation (9). Other reported activities of IL-4 on B cells, such as induction of cell adhesion (5) and motility (10, 11), suggest it can influence cell morphology. IL-4 has also been reported to directly affect cell morphology and cytoskeletal arrangement in human endothelial cells (12), providing a clear precedent for its ability to influence cytoskeletal organization. We report the ability of IL-4 to induce cell spreading and microvilli in B lymphocytes and discuss the physiologic significance of these findings.

Materials and Methods

Reagents and Abs

The following Abs were produced in the laboratory of D.H.C.: anti-mouse CD44 mAb RKC9 (13), an anti-DNP mouse IgE mAb (14), anti-mouse CD23 mAb B3B4 (15) and 4F8 (rat IgM), and polyclonal rabbit anti-CD23 antisera (IgG purified by protein G affinity chromatography; Pharmacia, Upplsa, Sweden). An additional anti-CD44 mAb KM201 was kindly provided by Southern Biotechnolate (Birmingham, AL) or purified from hybridoma supernatant. The hybridomas KM201 and FD448.1 secreting anti-LFA-1 αL-subunit mAb were purchased from the American Type Culture Collection (ATCC, Rockville, MD). Goat anti-mouse IgM polyclonal Ig was purchased from Dakopatts (Copenhagen, Denmark). Anti-IgM mAb Ak13 (16) was prepared by ammonium sulfate precipitation from culture supernatants and, where indicated, was used to couple to cyanogen bromide-activated Sepharose beads (Pharmacia) according to the manufacturer’s instructions (kindly prepared by Asta Miskiniene, Vilnius University, Vilnius, Lithuania). Soluble Ak13 was used at 10 μg/ml and the Sepharose-coupled Ab at 2% bead vol/vol. The hybridoma-secreting anti-CD40 mAb, IC10 (17), was generously provided by Maureen Howard (DNAX, Palo Alto, CA). IC10 mAb supernatants were ammonium sulfate precipitated and used at 10 μg/ml. The hybridoma producing a neutralizing mAb 11B11, recognizing mouse II-4, was kindly provided by Dr. William Paul (Bethesda, MD). Goat anti-rat IgG was obtained from Jackson Immunoresearch (West Grove, PA). A biotinylated B220 mAb, two mAbs recognizing the mouse αc integrin subunit (R1-2 and 9C10), and an anti-ICAM-1 mAb (3E2) were purchased from PharMingen (Hamburg, Germany). A

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Abbreviations used in this paper: VLA, very late antigen; IRS, insulin receptor substrate.

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hybridoma (Y11/17.4) producing a mAb recognizing ICAM-1 was ob-
tained from ATCC. Purified anti-mouse CD4 mAb, L3T4, was kindly pro-
vided by Dr. Anders Ön, Stockholm, Sweden. Streptavidin coupled to 
horseradish peroxidase or FITC were purchased from Dakopatts. Recom-
binant murine IL-4 was produced from IL-4 cDNA-transfected X63 cells 
(18), purified by passage through an 11B1 (anti-IL-4)-coupled column, 
and used at 5 × 10^6 units in all experiments. The amount of IL-4-induced 
ahalf-maximal response in induction of DNA synthesis in Con A-stimulated 
T cells is defined as 1 IU. IL-4 supernatant was used at the same unit 
activity in some experiments in which larger culture volumes were used.
LPS purified from Escherichia coli 055:B5 was provided by the Depart-
ment of Microbiology and Tumour Biology at Karolinska Institute and 
used at 25 μg/ml. Recombinant IL-2 and IL-5 were produced from X63 
cells transfected with IL-2 and IL-5 cDNA, respectively (18). IL-2 and 
IL-5 activity was determined in [3H]thymidine incorporation assays using 
CTLL or LyH7B13 cells, respectively, and were both used as supernatants 
added at two times the concentration giving half-maximal assay activity. 
Purified IL-10 was purchased from Genzyme, Cambridge, MA and used 
at 250 μg/ml. IL-10 at this concentration was able to inhibit IL-4-induced B 
cell proliferation (an indication of cell motility). Ref. 19) on freshly prepared 
B cells. Fibronectin was purified from fresh frozen human plasma by af-
finitivity chromatography on gelatin-Sepharose 4B (Pharmacia) and coated 
at a concentration of 10 μg/ml (a concentration used routinely to support 
attachment and growth of adherent cell lines). BSA fraction V was pur-
chased from Boehringer Mannheim (Mannheim, Germany). Other reagents 
were purchased from Sigma (St. Louis, MO).

Animals

C57BL/6 or CBA/J×C57BL/6F1 mice were used in all experiments. These 
were either bought from Bomholtgaard (Ny, Denmark), Charles River 
(Uppsala, Sweden), or bred and maintained in the animal facility at 
the Department of Cell and Molecular Biology, Karolinska Institute. 
CD23-deficient mice were kindly provided by Dr. Susanne Gustavsson 
(Uppsala, Sweden) (20). All experiments used age- and sex-matched 
animals.

Cell culture

Small resting B cells were prepared from splenic cell suspensions after T 
cell killing and Percoll gradient centrifugation as previously described (21). 
Cells were maintained in RPMI 1640 (Life Technologies, Paisley, Scot-
land) supplemented with 2 mM l-glutamine, 1 mM sodium pyruvate, 50 
IU/ml penicillin, 50 μg/ml streptomycin (Life Technologies), 50 μM 
2-ME, and 10% heat-inactivated FCS (Life Technologies) at 37°C in a 
humidified atmosphere containing 5% CO₂.

Cell morphology assays

B cells were cultured at 5 × 10⁵ per ml in the presence of various stimuli 
and for indicated times. Cells were resuspended by repeated pipetting and 
diluted to 10⁷ per ml in medium containing the original activating stimulus, 
transferred to Ab-coated plates, and cultured for the indicated periods. 
Ab coating of tissue culture plastic or glass cover slips was performed by 
adding 50 μg/ml of purified Ab in PBS including Mg²⁺ and Ca²⁺ and 
incubating for 1 h at room temperature. Plates were washed four times with 
Earle’s balanced salt solution (EBSS; Life Technologies, Paisley, Scot-
land) and supplemented with 2 mM l-glutamine, 1 mM sodium pyruvate, 50 
IU/ml penicillin, 50 μg/ml streptomycin (Life Technologies), 50 μM 
2-ME, and 10% heat-inactivated FCS (Life Technologies) at 37°C in a 
humidified atmosphere containing 5% CO₂.

Light microscopy

Cells were fixed with glutaraldehyde in RK3G9 (anti-CD44)-coated 24-
well plates and photographed using an inverted microscope and a ×40 
objective. Alternatively, cells were fixed with glutaraldehyde after cultur-
ing in 24-well plates on RK3G9-coated cover slips, stained with a biotin-
ylated anti-B220 (2 μg/ml) or an irrelevant biotinylated Ab followed by 
 streptavidin FITC (1/3000 dilution), and photographed with a ×100 oil 
immersion lens.

Electron microscopy

B cells were stimulated with LPS plus IL-4 or LPS for 2 days, transferred 
to anti-CD44-coated 9-cm tissue culture dishes at 2 × 10⁶ cells per ml, and 
cultured overnight. The medium was carefully removed from the plates 
and a primary fixation of 3% glutaraldehyde in 0.1 M sodium cacodylate-HCl 
buffer (pH 7.3) containing 0.05 M sucrose was applied. The cells were then 
removed by scraping and collected by centrifugation. Between 1 and 2 × 10⁶ 
cells were used per specimen. Postfixation was done for 2 h at 4°C in 
2% osmium tetroxide in 0.1 M cacodylate buffer (pH 7.3) containing 0.5% 
potassium ferrocyanate. The specimens were then dehydrated in graded 
ethanol (70–100%), stained with 2% uranyl acetate (in ethanol), and em-
bedded in Spurr low viscosity epoxy resin. Thin sections were cut on an 
LKB Instruments (Gaithersburg, MD) ultratome IV and examined in a 
JEOL (Tokyo, Japan) 100CX electron microscope at 60 kV.

Western blotting

B cells stimulated with LPS plus IL-4 or LPS alone for 3 days were har-
vested and resuspended in original medium at a cell density of 2 × 10⁶ 
cells per ml. These cells were then incubated for 2 h in the presence of 
anti-CD44 mAb RK3G9 (5 μg/10⁶ cells) or goat anti-rat IgG at 5 μg/ml 
(adDED for the final 90 min), or a combination of the two, or no Ab. Each 
determination used 10 to 40 × 10⁶ cells. After washing four times with 
EBSS containing 100 μM Na₂VO₃, cells were solubilized for 5 to 10 min 
at 4°C in a lysis buffer containing 0.5% Nonidet P-40, 150 mM NaCl, 10 
mM Tris (pH 7.6), 100 μM Na₂VO₃, 10 mM NaPO₄, 10 mM NaF, 1 mM 
PMSF, and 1 μg/ml respectively, of aprotinin and leupeptin. Lysates were 
centrifuged at 10,000 × g for 15 min. The cleared lysates were retained 
and the detergent-insoluble pellet was washed twice with lysis buffer and once 
in TBS. The detergent-insoluble pellets or 25 μg of detergent lysate were 
solubilized in nonreducing SDS-PAGE sample buffer and resolved on 7% 
SDS-PAGE gels. Western transfer to nitrocellulose (BA 83; Schleicher & 
Schuell, Dassel, Germany) was performed in 25 mM Tris, 192 mM glycine, 
and 20% methanol using a miniblot transfer chamber (Bio-Rad, Hercules, 
CA). The transfer conditions were 100 V (constant voltage) for 3 h at 4°C.

The filters were reversibly stained with 0.3% Ponceau S in 0.3% TCA 
(Serva, Heidelberg, Germany) to control for even transfer. Nonspecific 
protein binding sites were blocked by incubation for 20 min with blotting 
buffer containing 5% skimmed milk powder and 0.05% Tween-20 in 1 × 
TBS. The filters were stained with biotin-conjugated anti-CD44 mAb 
KM201 (1 μg/ml in blotting buffer for 1 h at 4°C) and washed four times 
with 0.05% Tween-20 in 1 × TBS. Streptavidin horseradish peroxidase 
conjugate was applied at a 1/1000 dilution in blotting buffer for 30 min and 
was washed as above. Detection was achieved by enhanced chemiluminescence 
using standard procedures and exposure to Fuji film.

Results

Cell spreading is induced in B cells stimulated with LPS plus IL-4

Murine B cells cultured in the presence of LPS plus IL-4 for 2 to 3 
days were previously reported to form large round cell 
aggregates. A low frequency of spread cells was consistently ob-
erved in such cultures. However, when cells were stimulated with 
LPS alone, neither spread cells nor regular large round aggregates 
were observed (5). We initially observed a high frequency of spread 
cells showing pronounced dendritic morphology in LPS 
plus IL-4-stimulated cultures containing anti-CD23 Abs and found this 
to be mediated by Abs bound to the tissue culture plastic 
(E.J.D. and E.S., unpublished observations). We have since made 
similar observations using a number of Abs deliberately immobi-
лизed to the tissue culture plastic. We used anti-CD44 mAb RK3G9 
extensively throughout this paper. Many cells bearing long and 
often branched dendritic projections were observed when B cells 
prestimulated with LPS plus IL-4 (Fig. 1a), but not LPS alone 
(Fig. 1b), were transferred to RK3G9-coated plates. LPS plus IL-
4-stimulated B cells were also found to spread on coverslips coated 
with RK3G9. Fluorescence staining with an anti-B220 mAb re-
vealed finely branched processes (Fig. 1c) not clearly seen with 
conventional phase contrast microscopy (Fig. 1d). Greater than
95% of spread cells were routinely found to be surface positive for B220, confirming that they were B cells and not a contaminant cell population.

IL-4 induction of dendritic morphology was dramatically reduced in the presence of anti-IL-4 mAb 11B11 (Fig. 2). When LPS blasts were recultured in the presence of LPS plus IL-4 on RK3G9-coated surfaces, 36% of cells were found to spread after 20 h. This was reduced to 7.6% when cells were similarly recultured in the presence of 10% 11B11 supernatant.

The kinetics of cell spreading

We investigated the kinetics of cell spreading to determine whether this correlated with other known B cell phenomena involving cell morphology. Early events in IL-4 signal transduction such as specific tyrosine phosphorylations occur within minutes of receptor engagement (22). Induction of cell polarization (an indication of cell motility) is clearly detectable 2 h after addition of IL-4 to primary B cells and maximal after 24 h (11), whereas homotypic B cell adhesion seen in LPS plus IL-4-stimulated cultures is maximal after 3 to 4 days (5). To study the kinetics of the spreading response, we plated freshly prepared B cells into RK3G9-coated wells and measured spreading at different time points. Figure 3A shows a response obtained within 20 h and that reached a maximum after 60 h of LPS plus IL-4 stimulation. Spreading was rarely observed in cultures before 6 h and fell after 3 days as cell death in cultures increased (not shown). B cells stimulated with either LPS or IL-4 alone or cultured in medium alone showed little tendency to spread. Thus, cell spreading appeared to correlate with blast formation in LPS plus IL-4-stimulated cultures. We were therefore interested in investigating whether late additions of IL-4 could induce spreading in LPS blasts and, if so, how rapidly this occurred. Day 2 LPS blasts were washed, resuspended in medium, and allowed to settle on RK3G9 wells in the presence of LPS, IL-4, combinations of the two, or no stimulus. Blasts restimulated with LPS plus IL-4 or IL-4 alone did not give a response that was different from that of LPS at 1 or 2 h time points, but did give a good response after 20 h (Fig. 3B). This shows that IL-4 induces cell spreading with slow kinetics.

Abs to diverse B cell surface structures are capable of mediating cell spreading

We were interested in investigating whether Abs to different cell surface determinants or other substrates could mediate a cell-spread response. In Table I we show that Abs to diverse surface structures, including CD23 (a C-type lectin and type II glycoprotein), CD44 (a proteoglycan), LFA-1, VLA-4 (both integrins), ICAM-1 (an Ig superfamily member), and membrane Ig, all supported cell spreading in the presence of LPS plus IL-4 (18–55% spread cells), while LPS blasts plated on the same Abs showed little tendency to spread (4.4% or less). We have yet to find an Ab that stains B cell blasts brightly in FACS analysis and fails to mediate cell spreading. A rat IgM anti-CD23 mAb (4F8) and a mouse IgE both supported spreading, suggesting that Fc receptor interactions are not essential. The surface expression of CD23 is greatly up-regulated on LPS plus IL-4-stimulated B cell blasts (13), possibly explaining the difference in the ability of LPS plus IL-4 and LPS blasts to spread. However, CD44 expression is increased after LPS stimulation but not further up-regulated in the presence of IL-4 (13), demonstrating that high surface expression is not a sufficient requirement for spreading. The IgE-mediated cell
we assume that this is due to a low affinity for fibronectin. and, as cells show little tendency to bind to the bottom of the wells, suggests that fibronectin is not capable of supporting this response from cells transferred to anti-CD44-coated plastic (Fig. 5). This ther at 2 or 20 h after transfer, while a high response was observed compared with those transferred to BSA-coated control wells, ei-

plus IL-4 failed to show any significant degree of spreading when coated with fibronectin, B cells prestimulated for 2 days with LPS port spreading of human B cells (8). When transferred to plates component, fibronectin, which has previously been shown to sup-

surfaces is indeed mediated by CD23. We wished to determine whether LPS plus IL-4-stimulated B cells are capable of spreading on a natural substrate. To do so, we chose an extracellular matrix

mediated by CD23 (FcεRI or the low affinity IgE receptor), the only known receptor for IgE on B cells. In Figure 4, we show that cells prepared from CD23-deficient mice were incapable of spreading on either B3B4 or IgE-coated surfaces while spreading normally on RK3G9-coated surfaces. Furthermore, when added to the medium at 20 μg/ml, B3B4 inhibited spreading of wild-type cells on B3B4 and IgE-coated surfaces, while not affecting that of RK3G9 coating. We conclude that B cell spreading on IgE-coated surfaces is indeed mediated by CD23. We wished to determine whether LPS plus IL-4-stimulated B cells are capable of spreading on a natural substrate. To do so, we chose an extracellular matrix component, fibronectin, which has previously been shown to support spreading of human B cells (8). When transferred to plates coated with fibronectin, B cells prestimulated for 2 days with LPS plus IL-4 failed to show any significant degree of spreading when compared with those transferred to BSA-coated control wells, ei-

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We next investigated whether other stimuli could induce cell spreading. Cells were stimulated for 2 days with anti-IgM reagents in the presence or absence of IL-4 and transferred to RK3G9-coated plates. In Table II, we see that such cells readily spread in the presence of IL-4 (17 and 22%, respectively, for anti-

IgM-coupled Sepharose and soluble Ab), albeit at a lower level than for LPS plus IL-4-stimulated cells (48%). No spreading was observed in the absence of IL-4. Anti-CD40-stimulated B cells

FIGURE 4. B cell spreading on IgE-coated surfaces is mediated by CD23. Day 2 LPS plus IL-4-stimulated B cell blasts prepared from spleens from wild-type (+/+ or CD23 deficient (−/−) mice were transferred to wells coated with RK3G9 (anti-CD44), B3B4 (anti-CD23), IgE mAb, or L3T4 (anti-CD4), respectively. Wild-type cells were also transferred to Ab-coated surfaces after 30 min of preincubation with 20 μg/ml B3B4 and incubated in the continuous presence of the Ab (marked +/+ plus sB3B4 in graph key). The cells were fixed after 20 h of incubation and cell spreading counted in duplicate. The data are the mean of two experiments.

<table>
<thead>
<tr>
<th>Table I. Ab mediating cell spreading*</th>
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<tr>
<td>Ab Coating</td>
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<tr>
<td>Experiment 1</td>
</tr>
<tr>
<td>RK3G9 (anti-CD44 mAb)</td>
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<tr>
<td>B3B4 (anti-CD23 IgG mAb)</td>
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<tr>
<td>Anti-CD23 (rabbit polyclonal)</td>
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<tr>
<td>4P8 (anti-CD23 IgM mAb)</td>
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<td>FK2.8 (anti-LFA-1 mAb)</td>
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<tr>
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</tr>
<tr>
<td>No Ab</td>
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<tr>
<td>Experiment 2</td>
</tr>
<tr>
<td>RK3G9 (anti-CD44 mAb)</td>
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<tr>
<td>KM201 (anti-CD44 mAb)</td>
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<tr>
<td>B3B4 (anti-CD23 IgG mAb)</td>
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<td>R1-2 (anti-VLA-4)</td>
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<tr>
<td>9C10 (anti-VLA-4)</td>
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<tr>
<td>YN1/1.7.4 (ICAM-1 mAb)</td>
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<td>3E2 (anti-ICAM-1)</td>
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<tr>
<td>L3T4 (anti-CD4 mAb)</td>
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<td>31M (anti-CD8 mAb)</td>
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* Small dense B cells were stimulated for 2 days with LPS and IL-4 or LPS alone. The cells were then resuspended to 10^6 per ml in medium plus stimulus, transferred to 96-well plates with appropriate Ab coating, and cultured overnight, after which they were fixed and duplicate determinations counted. The mean of three experiments is presented. n = 2 indicates Abs were used in two experiments only.
have previously been shown to form tight round cell aggregates (23, 24) reminiscent of those seen in LPS plus IL-4-activated cells (5). A high proportion of anti-CD40-stimulated cells spread in both the presence and absence of IL-4 (74 and 82%, respectively, as shown in Table II) and anti-CD40-induced cell spreading was not inhibited by a neutralizing anti-IL-4 mAb (11B11, Fig. 2), indicating that this phenomenon is not unique to IL-4-stimulated cells.

**IL-2, IL-5, and IL-10 fail to induce cell spreading**

IL-2, IL-5, and IL-10 all fail to induce cell spreading used alone or in combination with LPS (Table III). IL-10 has previously been reported to inhibit IL-4-induced B cell motility, and as this also involves alterations in cell morphology, we attempted to inhibit cell spreading with IL-10. However, IL-10 did not inhibit spreading induced by LPS plus IL-4.

**Electron microscopy reveals microvilli-like structures on LPS plus IL-4-stimulated blasts**

B cells cultured on RK3G9-coated surfaces after prestimulation with LPS plus IL-4 (Fig. 6, a and b) and LPS (Fig. 6, c and d) were examined by electron microscopy for structural features. Both groups displayed a ribosome-rich cytoplasm consistent with rapidly dividing blasts. Long cytoplasmic extensions generally devoid of membranous organelles were specifically found in the LPS plus IL-4-stimulated samples (not shown). Interestingly, microvilli-like structures were found frequently on LPS plus IL-4-stimulated cells (Fig. 6, a and b) and, while not being absent on LPS blasts, they are shorter and far less abundant in these cells (Fig. 6, c and d). Thus, IL-4 appears to influence B cell morphology at both a macroscopic and ultrastructural level.

**Interaction between CD44 and the cytoskeleton**

The dramatic changes in B cell morphology induced by LPS plus IL-4 stimulation suggest that a reorganization in the cytoskeleton takes place. The formation of dendritic morphology was found to be partially inhibited at 1 µM and totally inhibited at 10 µM cytochalasin B (Fig. 7), showing that it is dependent on intact actin cytoskeleton. Spread cells on Ab-coated surfaces were observed using video time-lapse photography. Some cells were anchored to the tissue culture plastic via the ends of dendritic processes, while the main body of cytoplasm moved back and forth between these points (not shown). These observations are consistent with a molecular mechanism of cell spreading including a direct linkage between the cytoskeleton and the membrane-attached protein. This would predict that surface molecules in LPS plus IL-4-stimulated B cells be anchored to the cytoskeleton. To address this issue, we looked for localization of CD44 in the insoluble fraction (containing the cytoskeleton) of detergent cell lysates. CD44 was initially chosen as it has been reported to interact with the cytoskeletal proteins (25, 26) and it is expressed equally on LPS plus IL-4- and LPS-stimulated B cell blasts (13). LPS or LPS plus IL-4-activated B cells were cross-linked with rat anti-mouse CD44 mAb and goat anti-rat Ig, solubilized with detergent, and the insoluble and soluble material were separated. As shown in Figure 8A, the detergent-insoluble fraction of the LPS plus IL-4-activated cells revealed a strong band of about 90 kDa (the m.w. of the common form of CD44). Significantly, weaker or no bands were detected in the case of LPS plus IL-4-stimulated cells treated with single or no Abs or similarly treated LPS blasts. In contrast, CD44 was always detected in the detergent-soluble fractions of cells treated in different ways, although it appears that LPS plus IL-4-activated cells express higher levels than did LPS-activated cells (Fig. 8B).

Additionally, we have examined LPS plus IL-4-stimulated B cells spread on anti-CD44 (RK3G9)-coated coverslips after paraformaldehyde fixation for localization or capping of CD44, as this would give a further indication of CD44 interaction with the cytoskeleton. However, while strong staining for CD44 was found over the entire cell, no specific localization was seen (not shown).

Taken together, the cytochalasin sensitivity of cell spreading and detergent-insoluble localization of CD44 suggest that...
cross-linking of CD44 induces attachment to the cytoskeleton in LPS plus IL-4 but not LPS-stimulated cells. We cannot exclude, however, that CD44 could instead associate with other molecules after IL-4 stimulation to produce a detergent-insoluble complex.

Discussion

Induction of an adherent or a motile cell phenotype frequently plays an important role in lymphocyte activation and is accompanied by changes in lymphocyte morphology. We describe here a novel function of IL-4 on murine B cells, in which it synergizes with LPS or anti-Ig reagents to induce cell spreading on adhesive surfaces to produce a striking dendritic morphology. A similar response is seen when B cells are stimulated with anti-CD40. While the physiologic significance of these observations still is unclear, they suggest a possible means by which IL-4 and CD40 may modulate immune responses modifying interactions of B cells with other cells or substrates.

IL-4 has previously been reported to induce cell motility (11, 27) and cell adhesion in B lymphocytes (5). It is therefore interesting to ask if the spreading phenomenon represents either of these activities. Many activating stimuli, including LPS (11) and CD40 ligation (3), in addition to IL-4 are capable of inducing cell motility in B cells. We have observed, using time-lapse photography, 2-day-stimulated LPS plus IL-4 and LPS B cell blasts (which differ greatly in their ability to spread) for motile behavior when cultured on thin collagen gels. Both blasts appear highly motile, exhibiting cytoplasmic movement and locomotion over the

![FIGURE 6.](image-url) Electron micrographs of B cell blasts from RK3G9-coated plates. Cells were stimulated with LPS plus IL-4 (a and b) or LPS alone (c and d). The scale bar represents 1 μm, N = nucleus, M = mitochondria, and G = Golgi complex. These observations are representative of two similar experiments. Induction of microvilli-like structures were observed consistently in at least four experiments using B cells cultured in suspension.

![FIGURE 7.](image-url) Cell spreading is sensitive to cytochalasin B. B cells were stimulated for 2 days with LPS plus IL-4 or LPS alone, exposed to cytochalasin B at indicated concentrations for 2 h, transferred to RK3G9-coated wells, and incubated for an additional 2 h. The cells were then glutaraldehyde fixed and spreading was counted in duplicate determinations. Data are the mean of three experiments and error bars represent SD.
The soluble and insoluble material were separated as indicated in Materials and Methods. Nonidet P-40-insoluble pellets were solubilized in nonreducing sample buffer, separated with SDS/PAGE, and Western blotted (A). Detergent-soluble lysates (25 μg) were similarly blotted (B). Bands were detected with anti-CD44 mAb KM201. Similar observations of CD44 localization in the detergent-insoluble fraction were made in at least five experiments, three of which included blots of detergent lysates.

Mitogen-induced homotypic B cell aggregation in vitro has been well documented (5, 24, 28, 29). Aggregation resulting in tight spherical cell clusters has been seen when cells are stimulated with LPS plus IL-4 (5), anti-Ig plus IL-4 (30), and anti-CD40 (which is often enhanced in the presence of IL-4) (24). We find that the same stimuli also induce cell spreading, whereas LPS-stimulated B cells form large loose cell aggregates and fail to spread. Cell spreading has similar slow kinetics as has the formation of tight aggregation, with both increasing slowly over 3 days. Taken together, this suggests that spreading and tight aggregation have mechanistic similarities.

Much progress has recently been made in understanding IL-4-mediated signal transduction. The IL-4 receptor can activate two signaling pathways, namely via the STAT 6 protein (31) and the insulin receptor substrate (IRS)-1 and -2 proteins (32). STAT 6 induces Ig class switching to IgG1 and IgE, and CD23 expression (33–35), while IRS-1 and -2 are heavily phosphorylated molecules that are capable of docking various signaling molecules via SH2 interactions and mediate an IL-4-dependent proliferative signal (36). We have shown that IL-2, IL-5, and IL-10 fail to substitute for IL-4 in our assay system, suggesting that an additional proliferative signal is not sufficient to induce this phenomenon. At present it is not known to what extent the STAT 6 and IRS-1 or -2 pathways contribute to morphology-related phenomena such as cell adhesion or cell motility. Interestingly, phosphatidylinositol-3 kinase has been implicated in platelet-derived growth factor-induced cytoskeletal rearrangements (37) and in IRS-1 signaling (22). Whether IL-4 can influence cell morphology by a phosphatidylinositol-3 kinase-dependent mechanism has yet to be determined.

The altered cell morphology described in this paper strongly suggests reorganization of the cytoskeleton. Furthermore, cell spreading mediated by immobilized Abs to plasma membrane molecules implicates an interaction between these molecules and the cytoskeleton. There are an increasing number of reports of interactions between plasma membrane proteins and the cytoskeleton.

Interestingly, CD44 is reported to interact with ankyrin (26) and the ERM family members ezrin, radixin, and moesin (25). Talin has been shown to colocalize with LFA-1 (38) and the β-1 integrin (as found in VLA-4) (39), ICAM-1 is reported to associate with α-actinin (40), surface Ig receptor is reported to localize in detergent-insoluble fractions (41), while there are no reports of CD23-cytoskeleton interaction. The regulation of such events is likely to be of importance in modulating events such as cell adhesion or signal transduction. Our observation that IL-4 can influence the detergent-insoluble localization of CD44 suggests that IL-4 may have such a regulatory role. We have also found that higher numbers of microvilli-like structures are induced on LPS plus IL-4-stimulated B cell blasts, compared with LPS blasts. The induction of microvilli is a complex process involving organization of microfilaments and their association with actin binding proteins such as ERM family members (42, 43) and provides further evidence that IL-4 can influence cytoskeleton organization. The microvilli-like structures have also been observed in LPS plus IL-4-induced aggregates (not shown), where they often make cell-cell contact, suggesting a role in cell adhesion. Involvement of microvilli in lymphocyte adhesion is not unprecedented, as microvilli have been reported on lymphocytes undergoing rolling in an L-selectin-dependent manner (44). The molecular nature of an IL-4-induced cytoskeleton-CD44 interaction and formation of microvilli are currently being investigated in our laboratory.

Cell spreading on immobilized Abs, while being useful for dissecting an interesting cellular response, is obviously an artificial system and raises the question of what these observations represent in vivo. The ability of two T cell-derived signals, namely IL-4 and CD40 ligation, to induce cell spreading strongly suggests the involvement of T cells. An interesting possibility is that the cell spreading we observe represents mechanisms involved in T cell-B cell collaboration. Early reports in which electron microscopy was used to examine Ag-specific interactions between B and T cells revealed extended areas of membrane contact between the two cell types and some attachments made via B cell microvilli (45). IL-4 has been observed to enhance the ability of Ag-specific B cells to form conjugates with T cells (46), suggesting a role for it in this process. More recent reports have shown that IL-4 is concentrated in T cells close to the B cell-contacting surface in an Ag-specific conjugate cell pair (47, 48). This provides a model for how a soluble factor like IL-4 can act in a cell-specific manner and enhance interaction between T and B cells. CD40 ligation, in addition to the spreading we observe, has been reported to induce strong homotypic B cell adhesion (23, 24). It is possible that signaling by CD40 ligand (gp39) expressed on activated T cells to B cells (49) results in enhanced Ag-specific interaction between the two cell types. We suggest that cytoskeletal changes induced by IL-4 and CD40 ligation make B cells bind more strongly to T cells, follicular dendritic cells, and/or other B cells within germinal centers.

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References

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