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# Interleukin-4-mediated aggregation of anti-IgM-stimulated human B cells: inhibition of aggregation but enhancement of proliferation by antibodies to LFA-1

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## SUMMARY

Direct cellular interactions, involving adhesion structures like lymphocyte function-associated antigen-1 (LFA-1) and intercellular adhesion molecule-1 (ICAM-1), play a critical role in the initial stages of T-cell-dependent B-cell activation. However, the relevance of cellular contact in later, lymphokine driven stages of B-cell stimulation is less well understood. We have here studied the ability of different lymphokines [interleukin (IL) -1$\alpha$, IL-1$\beta$, IL-2, IL-3, IL-4, IL-6 and interferon-$\gamma$ (IFN-$\gamma$)] to stimulate adhesion processes as well as proliferation of highly purified tonsillar B lymphocytes. None of the lymphokines were by themselves able to induce aggregation in resting B cells but, when added together with anti-IgM, IL-4 and to a lesser extent IL-2, promoted the formation of large, dense aggregates which were macroscopically visible after 3–4 days in culture. Addition of anti-LFA-1 antibodies (anti-CD11a or CD18) completely inhibited the lymphokine-promoted aggregation, indicating that cluster formation was mediated by LFA-1. Fluorescence-activated cell sorter (FACS) analysis showed that the expression of both LFA-1 and ICAM-1 increased after stimulation with IL-4 as well as with IFN-$\gamma$. However, in contrast to IL-4, IFN-$\gamma$ did not enhance cellular aggregation, suggesting that qualitative rather than quantitative changes in LFA-1/ICAM-1 promote aggregation. Although anti-LFA-1 antibodies inhibited aggregation of both IL-2- and IL-4-stimulated cells they did not inhibit proliferation. In contrast, in IL-4-stimulated cultures inhibition of cell contact resulted in a significantly increased proliferation. Furthermore, IFN-$\gamma$-stimulated cells responded with proliferation in the absence of aggregation. Taken together, the findings suggest that LFA-1-dependent cellular contact plays a minor role in lymphokine driven B-cell proliferation. The possible importance of aggregation in B-cell differentiation is discussed.


## INTRODUCTION

Interactions of lymphocytes by direct cell-to-cell contact is thought to be important in Th-cell-dependent B-cell activation.[1,2] These cellular interactions are dependent on adhesion molecules present on the surface of both resting and activated cells.[3] One of these molecules is LFA-1, a member of the integrin family.[4] LFA-1 is a heterodimer consisting of an $\alpha$-chain (CD11a) and a $\beta$-chain (CD18).[4] One of the ligands to LFA-1 is ICAM-1 (CD54) which is absent or weakly expressed on resting lymphocytes[5] but is induced during cellular activation.[6] Stimulation *in vitro* by the phorbol ester phorbol myristate acetate (PMA) causes rapid LFA-1-dependent homotypic aggregation of both B and T cells.[2] Moreover, many B-cell lines typically grow in large aggregates of which at least some have been shown to be LFA-1 dependent.[7]

Abbreviations: BSA, bovine serum albumin; FCS, fetal calf serum; ICAM-1, intercellular adhesion molecule 1; IL, interleukin; LFA-1, lymphocyte functional-associated antigen 1; PMA, phorbol myristate acetate.

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Although the importance of cell-to-cell contact in the early stages of T-cell-dependent B-cell activation is well established[1] it is still not clear whether cell contact also plays a role in the later, lymphokine-driven proliferative stages. We were therefore interested to see if any of several lymphokines (IL-1$\alpha$, IL-1$\beta$, IL-2, IL-3, IL-4, IL-6 and IFN-$\gamma$) were able to induce aggregation in purified B cells, whether this involved LFA-1, and whether aggregation was important for lymphokine-induced proliferation.

We here report that of the lymphokines tested, only IL-4 and to a lesser extent IL-2 were able to synergize with anti-IgM to stimulate aggregation in tonsillar B cells. This aggregation was visible after 3–4 days of culture and could be inhibited by anti-LFA-1 antibodies. In contrast, proliferation was either enhanced (IL-4) or not affected (IL-2) by the addition of these antibodies. The results indicate that proliferation of B cells may occur in the apparent absence of lymphokine-promoted cellular contact.

## MATERIALS AND METHODS

*Reagents and antibodies*

Recombinant IL-1$\beta$, IL-2, IL-3, IL-4, IL-6 and IFN-$\gamma$ were obtained from Genzyme, Boston, MA and IL-1$\alpha$ was a kind gift from Dr P. T. Lomedico, Hoffmann-La Roche Inc., Nutley, NJ. The biological activity of the various cytokines was confirmed by IL-1-induced production of IL-2 by the cell line LBRM 33 1A5 (ATCC CRL 8079, IL-1$\alpha$ and IL-1$\beta$), by proliferative response of bone marrow precursor cells (IL-3), by costimulation with anti-IgM beads (IL-2, IL-4, IFN-$\gamma$) and by IL-6-dependent growth of the mouse hybridoma cell line B45. Anti-IgM antibodies coupled to beads (Immunobeads) were from Bio-Rad, Richmond, CA. Neutralizing mouse monoclonal IL-6 antibodies (IgG1) were obtained from Genzyme and were used at doses which effectively blocked IL-6-induced growth of the B45-cell line. The anti-LFA-1 antibodies MHM23 (CD18) and MHM24 (CD11a) were purchased from Dakopatts, a/s, Copenhagen, Denmark and were used at 10 $\mu$g/ml. The anti-ICAM-1 (CD54) antibodies 7F7 and WEHI-ICAM were obtained from the 4th International Workshop and Conference on Human Leukocyte Differentiation Antigens.

*Cell preparation*

B cells were prepared from human tonsils as described previously.[8] Briefly, tonsils obtained from patients undergoing routine tonsillectomy (Danderyds Hospital, Stockholm, Sweden) were cut into fragments and dispersed into single-cell suspensions. T cells were removed by E rosetting followed by separation on Ficoll-Paque (Pharmacia, Uppsala, Sweden). The remaining cells were suspended in RPMI 1640 supplemented with 30% foetal calf serum (FCS) (Gibco, Paisley, Renfrewshire, U.K.) and monocytes were depleted by adherence to plastic tissue-culture flasks for 1 hr at 37°. The non-adherent cells were thereafter layered on a step gradient of 65, 52·5 and 42·5% Percoll (Pharmacia) and centrifuged for 10 min at 800 ***g***. The resting cells were collected from the 65–52·5% interphase and contained 96–98% CD20-positive cells (B1; Coulter Electronics, Hialeah, FL) and $\leqslant$1% CD3 or CD11b-positive cells (OKT3 and OKM1, respectively; American Type Culture Collection, Rochester, MD).

*Aggregation and proliferation*

All cultures were carried out in HEPES-buffered RPMI 1640 supplemented either with 10% FCS or 0·3% bovine serum albumine (BSA), penicillin (100 IU/ml), streptomycin (100 $\mu$g/ml), glutamine (2 mM) and $5 \times 10^{-5}$ M 2-mercaptoethanol (Serva Fein Biochemica, Heidelberg, Germany). B cells were cultured for 4 days at different cell densities ($0{\cdot}5$–$1{\cdot}5 \times 10^6$ cells/ml) in flat-bottomed multi-well tissue culture plates (Linbro Chemical Co., New Haven, CT) in a final volume of 0·2 ml/well. The cells were cultured with or without IL-1$\alpha$ or IL-1$\beta$ (final concentration 10 or 50 U/ml), IL-2 (0·1–1000 U/ml), IL-3 (500 or 1000 U/ml), IL-4 (0·1–1000 U/ml), IL-6 (100 or 1000 U/ml) or IFN-$\gamma$ (500 U/ml). The doses of lymphokines were chosen to be optimal as indicated by the manufacturer. Anti-IgM beads were used at a final concentration of 25 $\mu$g/ml. Cellular aggregation was estimated in an inverted microscope (Nikon, Yokohama, Japan) and scored according to a scale ranging from − to +++ reflecting the density as well as the size of the aggregates (Fig. 1). This subjective way of estimating aggregation was chosen since cellular proliferation had already taken place which made calculations of the percentage of free cells less decisive.

For determination of proliferation, cells were cultured for 84 hr and pulsed with [$^3$H]thymidine (1 $\mu$Ci/well, 18·0 Ci/mmol, Amersham, Amersham Bucks) during the final 18 hr and thereafter harvested onto glass fibre filter paper (Skatron, Lier, Norway). [$^3$H]thymidine incorporation was determined as triplicates in a liquid scintillation counter (LKB/Pharmacia).

*Flow cytometry*

For determinations of antigen expression cells were cultured at a density of $1 \times 10^6$ cells/ml in 24-well plates (Linbro) in the presence or absence of stimulating agents and stained on Days 0, 1, 2, 3 or 4 of culture. Cells were incubated with appropriately diluted mAb for 30 min at 4° and washed twice in Tris-buffered HBSS containing 0·02% sodium azide. After further incubation with fluorescein isothiocyanate (FITC)-conjugated antigen-binding fragment ($F(ab')_2$) of rabbit anti-mouse IgG (Dakopatts, a/s) the cells were investigated with a FACScan (Becton Dickinson, Mountain View, CA). Control cultures were stained with second-step antibodies only. When anti-IgM beads were present in the cell suspension, gates were set as to eliminate the beads.

## RESULTS

### IL-4 enhances aggregation of anti-IgM-stimulated human B cells

Highly purified resting tonsillar B cells were cultured with anti-IgM beads and/or IL-4. By itself, IL-4 induced a motile phenotype with dendritic morphology but did not cause cells to aggregate (Fig. 1b). Stimulation with anti-IgM beads in the absence of IL-4 led to a certain degree of aggregation (Fig. 1c). This aggregation was partly inhibited by low temperature and by sodium azide and occurred during the first 24 hr of culture. Interestingly, addition of IL-4 strikingly enhanced this aggregation (Fig. 1d) as well as induced proliferation. Aggregation was optimal on Day 4, occurred in both FCS and 0·3% BSA but not at 4° (data not shown). Delayed addition of IL-4 to anti-IgM-stimulated cells showed that optimal aggregation at Day 4 was obtained only if IL-4 was included during the first 24 hr of culture (data not shown). Although proliferation and aggregation were similar with regard to kinetics, enhanced aggregation required at least 10 U of IL-4/ml, whereas proliferation occurred already at 0·1 U/ml of IL-4 (Table 1). Cell density was also found to be an important parameter in aggregation, in as much as it increased extensively with a moderate increase in cell concentration. Thus, no significant aggregation was seen at $0{\cdot}5 \times 10^6$/ml whereas large compact clusters of cells were seen at cell concentrations exceeding $1{\cdot}0 \times 10^6$ cells/ml. On the other hand, proliferation appeared not to be directly correlated with cell density and increased only by a factor of two when the cell density was raised by a factor of three (see Fig. 3).

### Antibodies to LFA-1 inhibit aggregation but enhance proliferation induced by IL-4

To investigate the involvement of LFA-1 in the aggregation process, antibodies to the $\alpha$- (CD11a) or $\beta$-chain (CD18) of LFA-1(10 $\mu$g/ml of MHM24 or MHM23, respectively) were

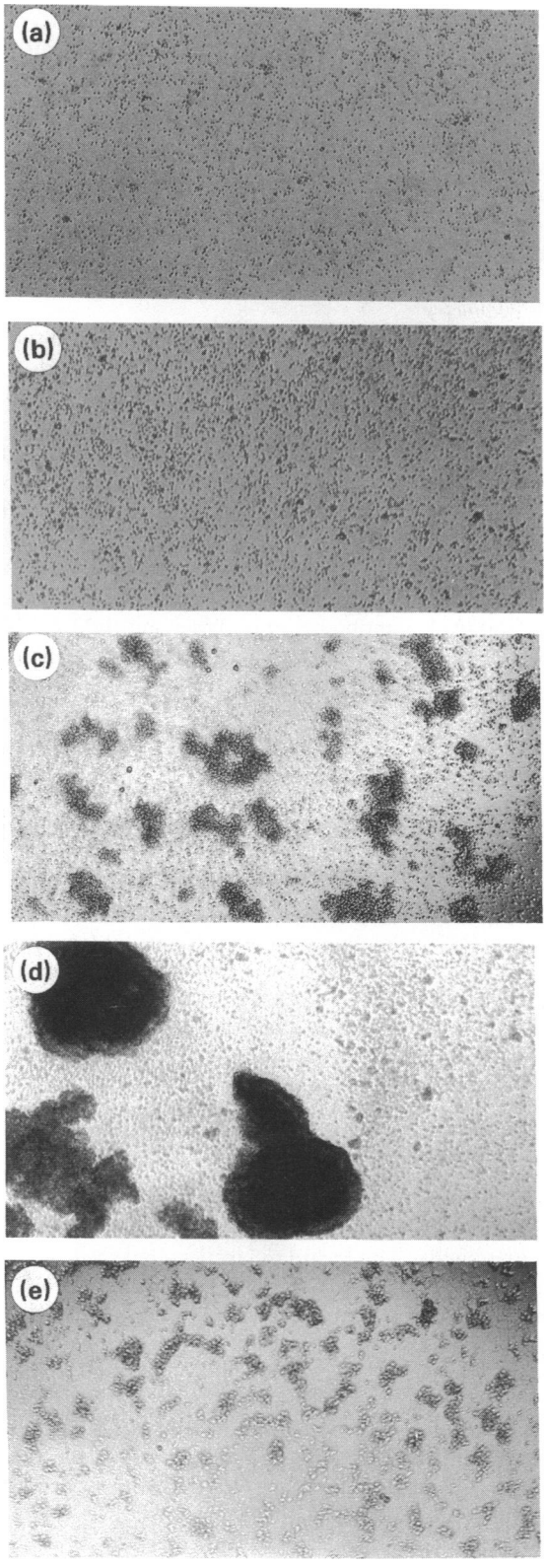


**Figure 1.** Effect of IL-4 and anti-IgM stimulation on homotypic B-cell aggregation. Resting tonsillar B cells ($1 \times 10^6$ cells/ml) were cultured for 4 days in (a) medium alone, (b) IL-4 (1000 U/ml) (c) anti-IgM (25 $\mu$g/ml), (d) a combination of IL-4 and anti-IgM, or (e) IL-4, anti-IgM and anti-CD18 antibodies (10 $\mu$g/ml of MHM23). Aggregation was estimated in an inverted microscope and scored as − (a, b), + (c) or + + + (d). Magnification ×40.

added to cultures of different cell densities. The IL-4-dependent aggregation was completely inhibited (Fig. 1e), irrespective of whether CD11a or CD18 antibodies were used (Table 2). However, anti-IgM-induced cluster formation was only marginally inhibited by the anti-LFA-1 antibodies, suggesting that adhesion mechanisms other than LFA-1/ICAM-1 were also involved. Interestingly, although anti-LFA-1 antibodies inhibited lymphokine-driven aggregation they did not inhibit proliferation. On the contrary, proliferation was significantly enhanced (Fig. 2) both in the presence of anti-CD11a or CD18 antibodies. This effect that was most evident at higher cell concentrations (Fig. 3).

### Effects of other lymphokines on aggregation/proliferation

Of six other lymphokines tested (IL-1$\alpha$, IL-1$\beta$, IL-2, IL-3, IL-6 and IFN-$\gamma$), only IL-2 promoted homotypic B-cell adhesion. As expected, IL-2 as well as IFN-$\gamma$, induced a significant degree of proliferation in anti-IgM-stimulated cells (see Table 4, data not shown). As with IL-4, aggregation with IL-2 was optimal on Day 4 and required simultaneous addition of anti-IgM to the cells (Table 3). However, aggregation with IL-2 (0·1–1000 U/ml) was not as strong as that observed with IL-4. As for IL-4, aggregation was inhibited by anti-LFA-1 antibodies. However, although LFA-1 antibodies inhibited aggregation they neither inhibited nor enhanced proliferation of IL-2-stimulated cells (Table 4).

As IL-4 has been found to induce IL-6 production we were interested to see whether endogenous IL-6 could synergize with anti-IgM and IL-4 in promoting aggregation. Neutralizing antibodies to IL-6 (20 $\mu$g/ml) were therefore added together with anti-IgM and IL-4. Interestingly, while these antibodies had no inhibitory affect on aggregation, they significantly enhanced proliferation ($P > 0{\cdot}05$, Table 5). To exclude the involvement of other autocrine factors, supernatants (25%) from Day 4 cultures of anti-IgM- and IL-4 stimulated cells were collected and added to freshly isolated cells alone or together with IL-4 and/or anti-IgM. No induction of aggregation occurred in the presence of these supernatants, nor did they change the kinetics of aggregation or proliferation in anti-IgM/IL-4-stimulated cells (data not shown).

### Enhanced expression of CD11a/CD18 and CD54 after stimulation with anti-IgM and IL-4 or IFN-$\gamma$

To study the expression of LFA-1 and ICAM-1 following IL-4 stimulation, cells were cultured for different time-periods and thereafter analysed with a FACScan. Anti-IgM or IL-4 alone had only marginal effects on the expression of the antigens (data not shown). However, stimulation by anti-IgM together with IL-4 increased the expression of LFA-1 as well as ICAM-1 (Fig. 4). As seen in Fig. 4a, CD11a and CD18 increased slightly on Day 2 and reached a maximum expression on Day 3. CD54, on the other hand, displayed a more rapid kinetics with an increase already on Day 1 and an optimal expression on Day 2 (Fig. 4b). Interestingly, at maximal aggregation on Day 4 the expression of this molecule had decreased to the level of Day 1.

In an effort to elucidate whether aggregation was caused merely by an upregulation of the adhesion molecules we also tested the effect of IFN-$\gamma$, a lymphokine which has previously been shown to induce CD54 expression on various cell types,

**Table 1.** Effect of IL-4 on B-cell aggregation and proliferation

| Stimuli | Aggregation | Proliferation (c.p.m.) | |
|---|---|---|---|
| | Exps 1 and 2 | Exp. 1 | Exp. 2 |
| Medium | – | 1232 ± 145 | 236 ± 18 |
| IL-4 (1000 U/ml) | – | 2494 ± 348 | 472 ± 51 |
| Anti-IgM (25 μg/ml) | + | 6993 ± 348 | 642 ± 184 |
| Anti-IgM + IL4 (1000 U/ml) | + + + | 74300 ± 2593 | 17138 ± 540 |
| Anti-IgM + IL4 (100 U/ml) | + + + | 49421 ± 7712 | 19102 ± 1006 |
| Anti-IgM + IL4 (10 U/ml) | + + | 27550 ± 4220 | 12385 ± 1019 |
| Anti-IgM + IL4 (1 U/ml) | + | 14453 ± 1300 | 7484 ± 223 |
| Anti-IgM + IL4 (0·1 U/ml) | + | ND | 3638 ± 793 |

**Table 2.** Inhibition of IL-4-induced aggregation by antibodies to LFA-1

| Stimuli | Aggregation Day 4 |
|---|---|
| Medium | – |
| Anti-CD11a (10 μg/ml) | – |
| Anti-CD18 (10 μg/ml | – |
| IL-4 (1000 U/ml) | – |
| IL-4 + anti-CD11a | – |
| IL-4 + anti-CD18 | – |
| Anti-IgM (25 μg/ml) | + |
| Anti-IgM + anti-CD11a | ± |
| Anti-IgM + anti-CD18 | ± |
| Anti-IgM + IL-4 | + + + |
| Anti-IgM + IL-4- + anti-CD11a | ± |
| Anti-IgM + IL-4 + anti-CD18 | ± |

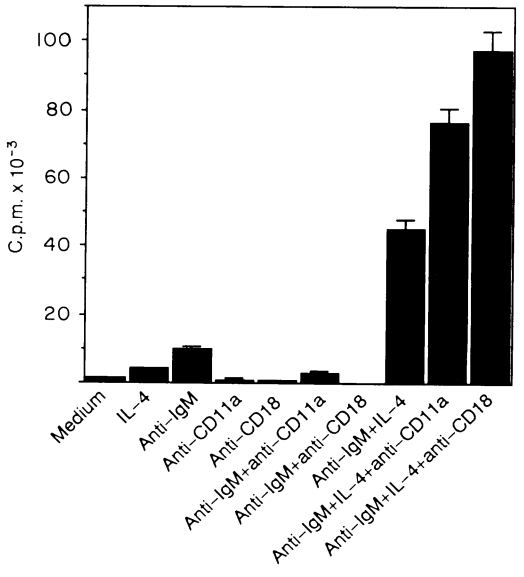


**Figure 2.** Effect of anti-LFA-1 antibodies on B-cell proliferation. Resting tonsillar B cells ($1{\cdot}5 \times 10^6$ cells/ml) were cultured with IL-4 (1000 U/ml), anti-IgM beads (25 μg/ml), anti-CD18 antibodies (10 μg/ml of MHM23) or combinations of IL-4, anti-IgM and anti-CD18 antibodies for 3 days and pulsed with [$^3$H]thymidine for 18 hr of culture.

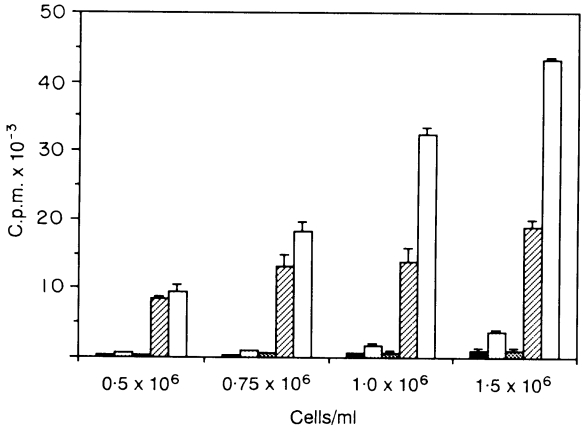


**Figure 3.** Effect of anti-LFA-1 antibodies on B-cell proliferation at different cell densities. Resting tonsillar B cells were cultured with medium alone (■) IL-4 (1000 U/ml) (▣), anti-IgM beads (25 μg/ml) (▣), IL-4 and anti-IgM beads (▨) or a combination of IL-4, anti-IgM and anti-CD18 antibodies (10 μg/ml of MHM23) (□) for 3 days and then pulsed with [$^3$H]thymidine for 18 hr.

**Table 3.** Aggregation of human B cells after stimulation for 4 days with different cytokines

| Stimuli | Without anti-IgM | With anti-IgM (25 μg/ml) |
|---|---|---|
| Medium | – | + |
| IL-1α (10 or 50 μg/ml) | – | + |
| IL-1β (10 or 50 μg/ml) | – | + |
| IL-2 (0·1–1000 U/ml) | – | + + |
| IL-3 (500 or 1000 U/ml) | – | + |
| IL-4 (0·1 1000 U/ml) | – | + + + |
| IL-6 (100 or 1000 U/ml) | – | + |
| IFN-γ (500 U/ml) | – | + |

**Table 4.** Effects of LFA-1 antibodies on IL-2-induced B cell-aggregation and proliferation

| Stimuli | Aggregation | Proliferation (c.p.m.) |
|---|---|---|
| Medium | – | 1188 ± 106 |
| IL-2 (20 U/ml) | – | 1804 ± 253 |
| IL-2 + anti-CD11a (10 μg/ml) | – | 1109 ± 426 |
| IL-2 + anti-CD18 (10 μg/ml) | – | 833 ± 456 |
| anti-IgM (25 μg/ml) | + | 7800 ± 1191 |
| anti-IgM + IL-2 | ++ | 81224 ± 8587 |
| anti-IgM + IL-2 + anti-CD11a | ± | 73820 ± 3839 |
| anti-IgM + IL-2 + anti-CD18 | ± | 78763 ± 2221 |

**Table 5.** Effect of IL-6 antibodies

| Stimuli | Exp. 1 | Exp. 2 | Exp. 3 |
|---|---|---|---|
| Medium | 99 ± 36 | 297 ± 44 | 199 ± 42 |
| Anti-IgM | 210 ± 39 | 1018 ± 230 | 2385 ± 164 |
| IL-4 | 802 ± 56 | 785 ± 177 | 1930 ± 324 |
| Anti-IgM + IL-4 | 21967 ± 2335 | 16783 ± 1254 | 20951 ± 1554 |
| Anti-IgM + IL-4 + anti-IL-6 | 28911 ± 2404 | 22574 ± 1713 | 23839 ± 1596 |

but as shown here, had no effect on cellular aggregation. Together with anti-IgM, IFN-γ upregulated not only CD54 but also CD11a and CD18 (Fig. 5). Whereas CD11a and CD18 expression followed the same kinetics as when the cells were cultured with anti-IgM and IL-4, CD54 expression had already reached a maximum on Day 1 and then slowly decreased. In spite of its ability to increase LFA-1 and ICAM-1, IFN-γ did not affect aggregation, indicating that the mere upregulation of the adhesion molecules is not sufficient to enhance cellular aggregation.

## DISCUSSION

In this study we show that IL-4 is able to synergize with anti-IgM to promote the formation of large, dense aggregates in highly purified B cells. Antibodies to the α- or β-chain of LFA-1 were able to inhibit the effect of IL-4 indicating that the effect was LFA-1 dependent. Furthermore, the process required energy, i.e. it did not occur at 4° or in cultures containing sodium azide (data not shown), observations consistent with an LFA-1-dependent mechanism.[4] Anti-IgM and IL-4 also promoted aggregation in serum-free cultures (0·3% BSA instead of FCS, data not shown), indicating that additional serum factors were not required. A similar but less pronounced aggregation was seen with IL-2 and anti-IgM but not with any of five other lymphocytes (IL-1α, IL-1β, IL-3, IL-6 and IFN-γ). The fact that optimal aggregation occurred on Day 4 of culture could implicate that another factor induced by IL-4 mediates aggregation. However, supernatants from stimulated cultures did not affect either aggregation or proliferation suggesting that IL-4 and anti-IgM by themselves were responsible.

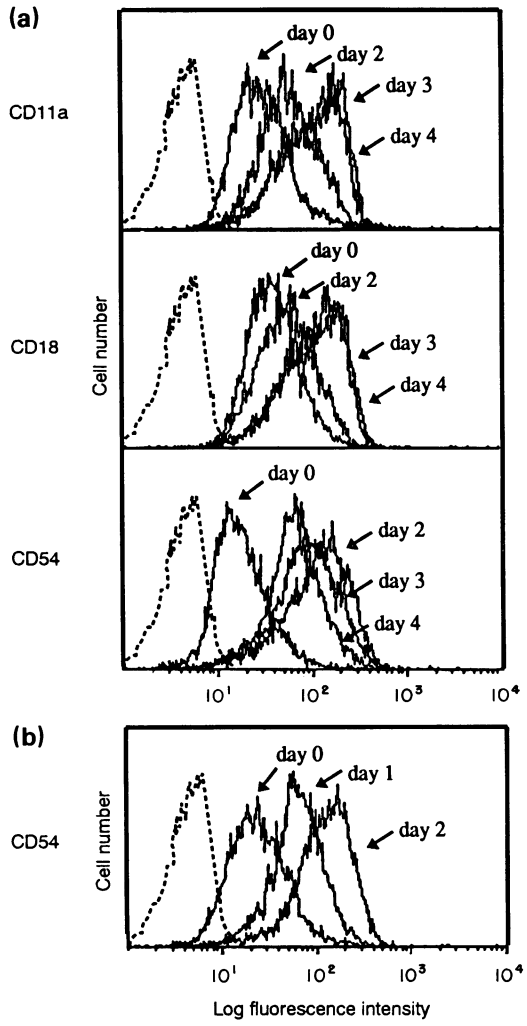


**Figure 4.** Kinetic analysis of CD11a, CD18 and CD54 expression. (a) FACS analysis of resting tonsillar B cells ($1 \times 10^6$ cells/ml) cultured for 0, 2, 3 or 4 days in the presence of IL-4 (1000 U/ml) and anti-IgM beads (25 μg/ml). Conjugate control (- - -) and staining of unstimulated cells were equal to Day 0 cells throughout the culture period. (b) CD54 expression on the same cells cultured for 0, 1 or 2 days in the presence of IL-4 and anti-IgM beads.

LFA-1-dependent homotypic B-cell aggregation has earlier been described for EBV-immortalized human B cells as well as for Burkitt's lymphoma cells.[2,9] Recently, antibodies to CD40 have been shown to induce aggregation which could be inhibited by LFA-1 antibodies.[10] However, B-cell aggregation promoted by IL-4 has to our knowledge only been described in the mouse system.[11] In the early stages of T-cell-dependent B-cell activation, addition of LFA-1 antibodies significantly inhibits T–B-interactions resulting in diminished B-cell proliferation.[1,12] It is still unclear however, whether cellular contact is also important in later proliferative stages of B-cell stimulation. Our finding that antibodies to LFA-1 did not inhibit IL-4- or IL-2-driven proliferation of anti-IgM-treated B cells suggests that cellular contact plays a minor role in these later stages. Since the LFA-1 antibodies did not completely abolish the aggregation it can be argued that the remaining clusters provided sufficient cell contact to allow proliferation. However, we think that this is less likely since most, if not all, of this aggregation could be

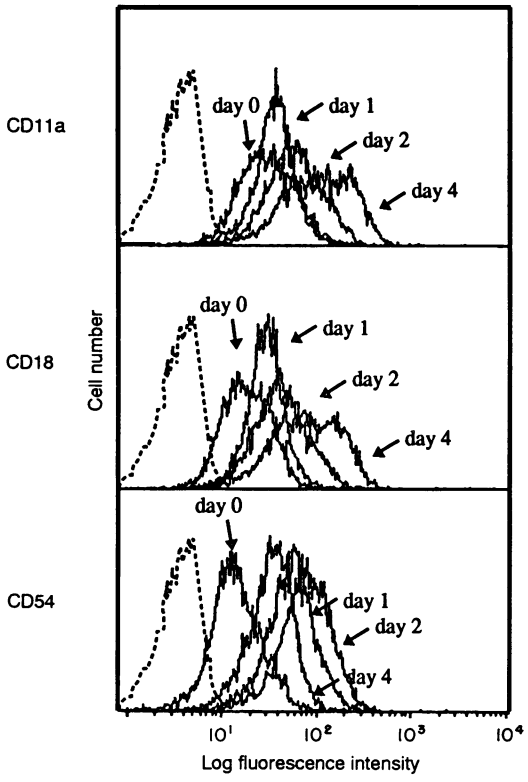


**Figure 5.** Kinetic analysis of CD11a, CD18 and CD54 expression. FACS analysis of resting tonsillar B cells ($1 \times 10^6$ cells/ml) cultured for 1, 2 or 4 days in the presence of IFN-$\gamma$ (500 U/ml) and anti-IgM beads (25 $\mu$g/ml). Conjugate control (- - -) and staining of unstimulated cells were equal to Day 0 cells throughout the culture period.

attributed to a passive agglutination of the cells with the IgM beads. Furthermore, we have recently observed that IL-4 together with antibodies to CD40 induce strong aggregation that can be completely blocked by anti-LFA-1 antibodies without inhibiting cell proliferation (P. Björck and S. Paulie manuscript in preparation). Interestingly, at cell densities exceeding $0{\cdot}75 \times 10^6$ cells/ml, addition of LFA-1 antibodies not only did not inhibit but significantly enhanced the anti-IgM + IL-4-induced proliferation. It appears unlikely that this enhancement was due to the delivery of a positive signal via the LFA-1 molecule, since no such effect of anti-LFA-1 antibodies was seen at lower cell densities. A simple explanation for the enhanced proliferation could be that cells located in the centre of the large clusters suffer from a lack of nutrients. Thus, a lesser degree of aggregation would then provide these cells with a microenvironment enabling optimal proliferation. Another possibility would be that anti-LFA-1 antibodies increase proliferation by reducing close cell contact and thus the efficiency of one or more differentiation factors triggered in response to IL-4. Such a factor could be IL-6, which is known to be produced by IL-4-stimulated cells[13] and which together with IL-4 induces differentiation.[14] In line with this hypothesis is that addition of IL-6 antibodies to anti-IgM- and IL-4-stimulated cultures significantly increased proliferation.

We found that IL-4 increased the expression of LFA-1 and ICAM-1 molecules. It has earlier been shown that IL-4 and anti-IgM can upregulate CD11a but not CD18 on tonsillar B cells.[15] However, we found that both antigens were upregulated in parallel, which might be expected with regard to the dimeric structure of the molecule. The fact that upregulation and aggregation occurred simultaneously implies that these events may be causally related. However, as shown here, IFN-$\gamma$ had a similar effect as IL-4 on the expression of the LFA-1/ICAM-1 antigens but did not induce aggregation. This suggests that qualitative rather than quantitative changes in the adhesion molecules are important in the events leading to aggregation.

Although the physiological role of homotypic B-cell aggregation is unknown, the fact that IL-4, as well as IL-2, induced this type of aggregation indicates that it has an important role in B-cell development. Rather than being associated with proliferation, homotypic cell contact may be important in differentiation events in the lymph node follicles.

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