Regulated Expression and Function of CD11c/CD18 Integrin on Human B Lymphocytes. Relation between Attachment to Fibrinogen and Triggering of Proliferation through CD11c/CD18

By Antonio A. Postigo, Angel L. Corbí,* Francisco Sánchez-Madrid, and Manuel O. de Landázuri

*From the Servicio de Inmunología and *Unidad de Biología Molecular, Hospital de la Princesa, Universidad Autónoma de Madrid, Madrid, Spain*

Summary

CD11c/CD18 (p150,95) is a β2 integrin expressed by myeloid, natural killer and certain lymphoid cells such as some cytotoxic T cell clones and B cell malignancies. We have studied the expression and function of CD11c on resting and activated B lymphocytes. Flow cytometry, immunoprecipitation, and mRNA analyses showed that cell activation with phorbol esters or with a variety of stimuli such as *Staphylococcus aureus* or anti-μ antibodies in combination with cytokines induced de novo CD11c/CD18 cell surface expression on most B cells while CD11b expression was not affected. Functional analysis of CD11c/CD18 on B cells revealed that it plays a dual role. First, CD11c/CD18 is implicated in B cell proliferation, as demonstrated by the ability of several anti-CD11c monoclonal antibodies to trigger comitogenic signals; and second, the newly expressed CD11c/CD18 mediates B cell binding to fibrinogen. Our data conclusively demonstrate the role of CD11c/CD18 on both B cell activation and adhesion processes.

The integrin family is composed by at least 15 cell surface heterodimeric glycoproteins that function in cell-cell contacts and cell-extracellular matrix interactions (reviewed in references 1–5). Although originally believed to participate only in adhesion processes, recent studies indicate that integrins are also involved in cell activation phenomena (6–15).

Integrins have been divided in three subfamilies that can be distinguished by their common use of a unique β chain noncovalently associated with several distinct α chains. p150,95 (CD11c/CD18), together with LFA-1 (CD11a/CD18) and Mac-1 (CD11b/CD18), belongs to the β2 integrin subfamily whose expression is restricted to leukocytes. While LFA-1 is expressed on all leukocytes, Mac-1 and p150,95 are expressed mainly by granulocytes, monocytes and NK cells (16, 17). Moreover, CD11c/CD18 is present on dendritic cells (18, 19), certain cytotoxic T cell clones (20), and some lymphocytes of the B cell lineage like those of the hairy cell leukemia (HCL)[1] (21). Coexpression of Mac-1 and p150,95 by some B cell chronic lymphocytic leukemias (B-CLL) has also been reported (22). CD11c/CD18 has been described participating in a number of adhesion phenomena such as binding to the iC3b or fibrinogen and attachment to endothelium (reviewed in reference 3).

During activation, B cells undergo a number of changes on the expression of their different surface antigens (23–32). Since Mac-1 and p150,95 are expressed by certain transformed B cells, we explored whether these two integrins could be induced during in vitro activation of normal B cells. The data reported here demonstrate that cell surface expression of CD11c/CD18, but not CD11b/CD18, is induced during activation of purified B lymphocytes. The involvement of CD11c/CD18 on B cell activation is highlighted by the capacity of CD11c mAbs to trigger comitogenic signals on B lymphocytes. In addition, the expression of CD11c/CD18 also confers fibrinogen binding ability to B cells.

Materials and Methods

Monoclonal Antibodies. BU12 mAb (anti-CD19) was generously provided by Dr. Johnson (University of Birmingham, UK). 1F5 mAb (anti-CD20) was a gift from Dr. Clark (University of Washington, Seattle, WA). HB5 mAb (anti-CD21) was generously provided by Dr. Cooper (La Jolla, CA). HC1/1 mAb (IgG1) directed to α chain of p150,95 was obtained in our laboratory (33). Other CD11c mAbs were kindly provided by Dr. Johnson (BU15 mAb, IgG1), Dr. Lanier (DNAX, Palo Alto, CA) (L29 mAb, IgG1), Dr. Pulford (University of Oxford, UK) (KB23 and KB90 mAbs), and

[1] *Abbreviations used in this paper:* Ab, antibody; BCLL, B cell chronic lymphocytic leukemias; FG, Fibrinogen; HCL, hairy cell leukemia; SAC, *Staphylococcus aureus* Cowan I.

Dr. Schwarting (Thomas Jefferson University, Philadelphia, PA) (S-HCL 3 mAb, IgG2a). TS1/11 mAb directed to CD11a chain, TS1/18 mAb against the β2 integrin, and Bear 1 mAb directed to CD11b molecule were previously described (34, 35). TS2/18 mAb is directed against the CD2 antigen. Anti-CD69 TP1/8, anti-CD45 D3/9, and anti-CD49d (VLA-α4) HP2/1 mAbs were obtained in our laboratory (36–38). $F(ab')_2$ fragments of HC1/1 mAb were obtained upon pepsine treatment as described (39).

Cells and Cell Cultures. Tonsillar B cells were obtained from children 4–12 yr old undergoing routine tonsillectomy. Mononuclear cells were isolated by Ficoll-Hypaque (Pharmacia Fine Chemicals, Uppsala, Sweden) centrifugation and enriched for B cells by rosetting with 2-aminoethyl-isothiouronium bromide (AET) (Sigma Chemical Co., St. Louis, MO)-treated sheep erythrocytes (Biomerieux, Marcy-L'Etoile, France). The resulting B cell–enriched population was >95% $CD19^+$ and $CD20^+$, and <2% $CD11b^+$ and $CD2^+$.

In some experiments, tonsillar B cells were separated over a Percoll (Pharmacia Fine Chemicals) discontinous gradient on five bands that layered from medium to 45%, 45–55%, 55–65%, 65–75%, and 75–100%. Then, each fraction was activated and assayed for flow cytometry.

Peripheral blood B cells were obtained from heparinized venous blood of normal voluntary donors. After Ficoll-Hypaque centrifugation, peripheral mononuclear cells were depleted of adherent cells by two steps of adherence incubation in plastic flasks (Costar, Cambridge, MA) at 37°C and 5% CO_2 atmosphere for 2 h. T cell depletion was accomplished by removing the cells that rosetted with AET-treated sheep erythrocytes as described above. Cells were grown in RPMI 1640 (Whittaker M.A. Bioproducts, Walkersville, MD) supplemented with 10% FCS (Biochrom; Seromed, Berlin, Germany), 2 mM L-glutamine (Whittaker M.A. Bioproducts), 50 U/ml of penicillin, and 50 μg/ml of streptomicin (Whittaker M.A. Bioproducts). This medium will be referred as complete medium.

Reagents. Human rIL-2 and rIFN-γ was a generous gift from Hoffman-La Roche, Inc. (Nutley, NJ). Human rIL-4 was kindly provided by Dr. J. de Vries (Unicet Labs., Dardilly, France) and human rTNF-α by Labs. Andrómaco (Madrid, Spain). PMA, phorbol 12,13 dibutyrate (PDB), and $F(ab')_2$ fragments of rabbit polyclonal antibodies to human μ chain were purchased from Sigma Chemical Co. *Staphylococcus aureus* strain Cowan I (SAC) (Pansorbin) was acquired to Calbiochem-Behring Corp. (San Diego, CA). Cytokines are used at the following doses: rIL-2, 50 U/ml; rIL-4, 600 U/ml; rTNF-α, 100 ng/ml; rIFN-γ, 200 U/ml. SAC was used at a $1/10^4$ (vol/vol) dilution. Anti-μ antibodies were coated to plastic plates at 50 μg/ml in PBS, pH 8.0, overnight at 4°C.

Flow Cytometry Analysis. $1.5–2 \times 10^5$ cells were incubated with 100 μl hybridoma culture supernatants for 30 min at 4°C. After two washes with cold PBS, the cells were stained with a goat anti-mouse $F(ab')_2$ fragment FITC (Dakopatts, Glostrup, Denmark) followed by two additional washes with cold PBS. Cell immunofluorescence was analyzed by flow cytometry using a EPICS-C (Coulter Immunology, Hialeah, FL). Fluoresceined microspheres of different intensities were analyzed in both log and linear scale, and a standard conversion scale was constructed. Cell fluorescence data were collected on the log scale and converted to the linear scale for a quantitative estimation. Specific relative linear fluorescence was obtained by subtracting background linear fluorescence produced by the negative control myeloma P3X63. The percentage of positive cells was calculated by subtracting the percentage of control X63 from that obtained with every specific marker.

^{125}I Radiolabeling, Immunoprecipitation, and Electrophoresis. Tonsillar B lymphocytes and granulocytes were radioiodinated with tetrachloro-diphenil-glycoluril (Iodogen; Sigma Chemical Co.) and 1 mCi of ^{125}I (DuPont Co., Wilmington, DE) each 2×10^7 cells. Cells were lysed in a buffer lysis containing 1% NP-40 (Sigma Chemical Co.), 10 mM Tris-HCl, pH 7.4, 0.15 M NaCl, 1% BSA, 1 mM PMSF (Sigma Chemical Co.), 1 mM EDTA, and 50 mM NaF. Equal amounts of input radioactivity of ^{125}I-labeled cell lysates (10^6 cpm/condition) were incubated with 100 μl of mAb culture supernatants. Immunecomplexes were isolated by incubation with 100 μl of 187.1 anti-mouse κ chain mAb followed by incubation with 30 μl of protein A from *S. aureus* coupled to Sepharose (CL-4B; Pharmacia Fine Chemicals). Immunoprecipitates were processed as described elsewhere. Samples were resolved in 7% SDS-PAGE gels and autoradiographed.

RNA Blot Hybridization. Total RNA from tonsillar B cells and the U937 cell line was prepared by the guanidinium thiocyanate/cesium chloride method. 10 μg/lane was loaded on formaldehyde-agarose gels, subjected to electrophoresis, and transferred to nitrocellulose filters (Bio-Rad Laboratories, Richmond, CA). Blots were hybridized with ^{32}P-labeled 0.8-kb PstI CD11c probe (40) and a 1-kb EcoRI CD18 fragment (41).

Proliferation Assays. Purified tonsillar B lymphocytes at 1.5×10^6 cells/ml were added in 100 μl of complete medium to flat-bottomed 96-well culture plates (Costar). The different stimuli and mAbs were added each in 10 μl and wells were completed with RPMI 1640-10% FCS to reach 200 μl. Plates were incubated for 72 h at 37°C in a 5% CO_2 atmosphere. In proliferation assays with crosslinked mAb, wells were coated with purified mAb at different concentrations. After overnight incubation, plates were gently washed with RPMI 1640 and cells were added. Cell proliferation was estimated by $[^3H]TdR$ (DuPont Co.) incorporation during the last 18 h of culture. Cells were harvested in a cell harvester (Skatron, Lier, Norway), and the radioactivity was measured in a liquid scintillation β counter (Kontron, Zurich, Switzerland).

Cell Attachment Assays. Fibrinogen and type I collagen were purchased from Sigma Chemical Co. A 38-kD proteolytic fibronectin fragment was kindly provided by Dr. A. García-Pardo (Centro Investigaciones Biológicas, Madrid). 96-well microtiter EIA II Linbro plate (Flow Laboratories, Inc., McLean, VA) were coated overnight at 4°C with 100 μl of different proteins dissolved in CO_3HNa (0.1 M) at 20 μg/ml and then saturated with RPMI 1640-1% HSA for 2 h at 37°C. Thereafter, plates were washed gently with medium and 1.5×10^5 cells/well in 100 μl were added and incubated for 30 min at 37°C. After incubation, plates were washed three times and examined in an inverted microscope by at least two independent observers. Each condition was performed on duplicate. Cells were counted from at least three fields within each well. The number of cells counted in a nonwashed well was referred as input cells. In inhibition assays, cells were incubated for 30 min at 4°C with the different mAbs and then added to wells.

Results

Induction of CD11c Expression after B Cell Activation. Flow cytometry was used to study the cell surface expression pattern of the β2 integrin family during B cell activation. As previously described, nonactivated B cells highly expressed CD11a and CD18, whereas no significant expression of CD11b (<1%) or CD11c (<5%) was detected (Fig. 1). To ascertain whether B cell activation could alter the expression of β2 integrins, purified tonsillar B cells were treated with PMA. Phorbol ester treatment induced an increase in CD11a and

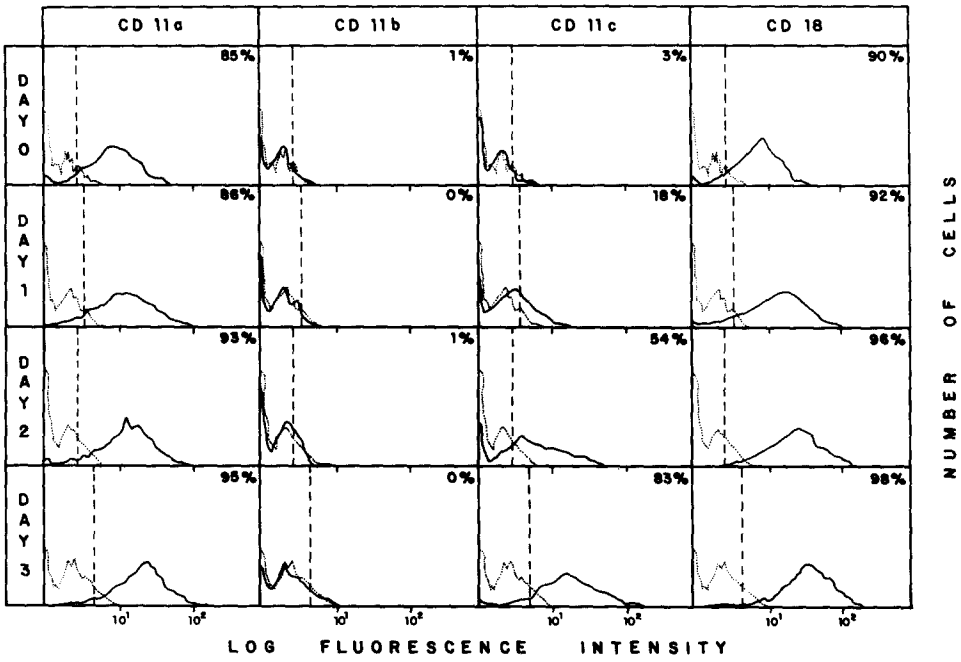

Figure 1. Expression of $\beta 2$ integrins on nonactivated and activated tonsillar B cells. Immunofluorescence flow cytometry analysis was performed on tonsillar B lymphocytes either nonactivated or cultured for several days in the presence of PMA (5 ng/ml). Cells were labeled with TS1/11 mAb (CD11a), Bear 1 mAb (CD11b), HC1/1 mAb (CD11c), or TS1/18 mAb (CD18) (*solid lines*), and with the negative control X63 (*dotted line*). In a second step, the cells were stained with a goat anti-mouse IgG FITC. Cell immunofluorescence was analyzed in an EPICS-C. *x*-axis, log fluorescence intensity; *y*-axis, frequency.

CD18 expression without significant changes in the percentage of positive cells. On the contrary, PMA elicited a gradual induction in the expression of CD11c and, at day 3, most B cells became CD11c$^+$. The percentage of CD11c$^+$ cells remained constant at days 4 and 5 (Fig. 1, and data not shown). No concomitant increase of CD11b expression was detected under the same conditions. This selective CD11c induction was not restricted to a particular tonsillar B cell subpopulation as shown by Percoll gradient separation (see Materials and Methods), and was also observed on purified peripheral blood B cells (data not shown).

Next, we investigated whether the PMA-induced CD11c expression could be altered by other stimuli each as the polyclonal activator SAC, rIL-2, rIL-4, rIFN-γ, rTNF-α, or anti-μ antibodies (Ab). PMA-induction of CD11c/CD18 was significantly enhanced by SAC, rTNF-α, or polyclonal anti-μ Ab (data not shown). Similarly, CD11c expression could also be obtained after treatment with more "physiological" B cell activation agents: SAC or anti-μ Ab combined with cytokines were able to induce CD11c expression, although to a lesser extent than phorbol esters (Table 1).

The induction of CD11c expression was studied at the biochemical level by immunoprecipitation from both activated and nonactivated tonsillar B cell lysates with mAbs against the different members of the $\beta 2$ integrin family (Fig. 2). While only CD11a and CD18 glycoproteins were precipitated from nonactivated B cells (Fig. 2 *A*), immunoprecipitation from 3-d phorbol ester–activated tonsillar B lymphocytes revealed the presence of CD11c/CD18, with its corresponding polypeptides of 150 and 95 kD (Fig. 2 *B*), thus confirming the cell surface expression of this heterodimer on activated B cells.

On neutrophils, activation is followed by a rapid increase in the cell surface expression of Mac-1 and CD11c, due to

Table 1. *Induction of CD11c on 3-d-activated Tonsillar B Cells by SAC, anti-IgM, and Cytokines*

Stimulus	Percent of positive cells	Specific relative fluorescence
Control	2	1
rIL-2	1	3
rIL-4	16	10
rTNF-α	3	2
rIFN-γ	2	1
SAC	18	14
SAC + rIL-2	22	15
SAC + rIL-4	33	19
SAC + rTNF-α	31	18
SAC + rIFN-γ	23	15
SAC + rTNF-α + rIL-4	45	25
SAC + rIFN-γ + rIL-2	20	13
Anti-μ	16	12
Anti-μ + rIL-2	23	15
Anti-μ + rIL-4	30	19
Anti-μ + rTNF-α	27	17
Anti-μ + rIFN-γ	19	14

Expression of CD11c on tonsillar B cells cultured for 3 d with different stimuli (see Materials and Methods). Control represents the culture of B cells in complete medium. Specific relative fluorescence and percentage of positive cells were calculated as described in Materials and Methods. Data represent one experiment out of five different assays.

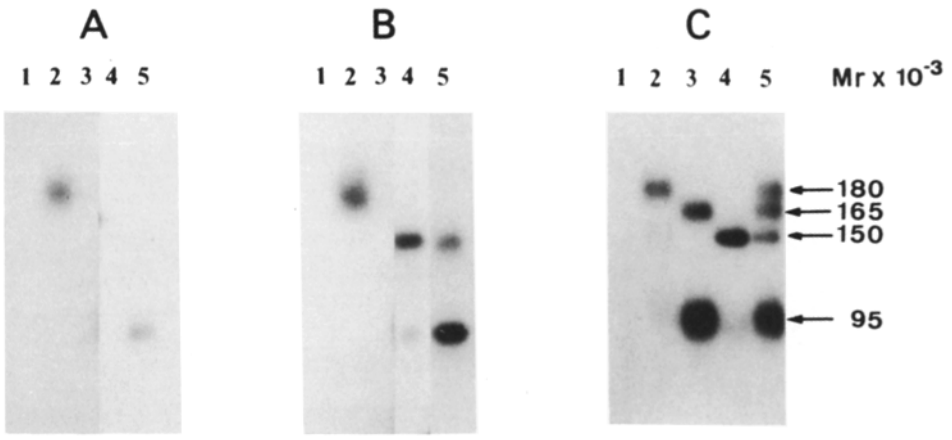

Figure 2. Immunoprecipitation of CD11/CD18 antigens from nonactivated (*A*) and activated (*B*) tonsillar B cells and from granulocytes (*C*). (*B*) B lymphocytes were subjected for 3 d to 5 ng/ml of PMA. ^{125}I cell lysates were immunoprecipitated with control P3X63 mAb (lane *1*), anti-CD11a TS1/11 mAb (lane *2*), anti-CD11b Bear 1 mAb (lane *3*), anti-CD11c HC1/1 mAb (lane *4*), and anti-CD18 TS1/18 mAb (lane *5*). Reduced samples were subjected to a 7% SDS-PAGE.

the release of a preformed pool from intracellular granules (reviewed in reference 3). To analyze the mechanisms of induction of CD11c/CD18 B cell surface expression, the steady-state level of mRNA for both chains was determined by Northern blot. While no mRNA for CD11c was detected on resting B cells, CD11c mRNA was evident at day 2 and reached a maximum by day 3 (Fig. 3 *A*). CD18 mRNA was already present on resting B cells (as expected from their LFA-1 [CD11a/CD18] constitutive expression) and also showed an increase after PMA treatment (Fig. 3 *B*). The kinetics of the CD11c/CD18 appearance on the membrane and the mRNA levels for CD11c and CD18 suggest that the induced cell surface expression of CD11c/CD18 is not due to the release of a preformed pool but probably to the activation of the transcription of the CD11c gene and/or to an increase in the CD11c mRNA messenger stability.

Monoclonal Antibodies Directed to CD11c Trigger Proliferative Responses on Activated Human B Lymphocytes. The expression of CD11c on activated B cells suggested that it could play a role in the regulation of B cell function at this stage. Since a number of integrins have been shown to trigger comitogenic signals on T cells (6–15), the functional effect of a wide panel of CD11c mAb was tested in proliferation assays. Highly purified tonsillar B cells were cultured with PMA to induce CD11c expression and were treated with CD11c mAb. Three CD11c mAbs induced comitogenic responses ranging from 2.5- to 8-fold higher than PMA alone (Fig. 4). HC1/1 and KB23 mAbs triggered lower mitogenic responses (2.5–4-fold) than BU15 mAb (4- to 8-fold). This CD11c-mediated proliferative response was comparable to those observed with other stimuli or mitogenic combinations such as PMA plus anti-CD69 TP1/8 or anti-CD20 1F5 mAb and rIL-2 (or rIL-4) plus PMA (Fig. 4; and data not shown). The anti-CD21 HB5 mAb or the anti-CD45 D3/9 mAb, included as controls, did not affect the response induced by PMA (Fig. 3; and data not shown). Other anti-CD11c mAbs such as L29, KB90, and S-HCL 3 failed to alter the PMA-induced proliferation (Fig. 4; and data not shown).

The CD11c mAb–mediated B cell proliferation was shown to be: (*a*) dose dependent for both mAb and phorbol ester (Fig. 5); (*b*) isotype independent, as concluded from the Ig class of the mAb tested; and (*c*) Fc independent, since similar proliferation effects could be obtained with the $F(ab')_2$ fragments of HC1/1 mAb (Figs. 4 and 5). In addition, cross-

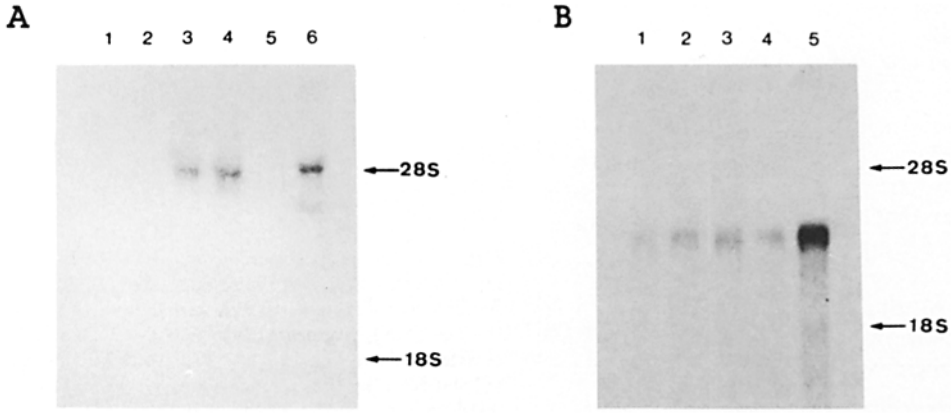

Figure 3. Induction of CD11c/CD18 mRNA levels in purified human tonsillar B lymphocytes. (*A*) CD11c mRNA levels in B lymphocytes either nonactivated (lane *1*), or PMA-activated (5 ng/ml) for 24 h (lane *2*), 48 h (lane *3*), and 72 h (lane *4*). mRNA from the myelomonocytic U937 cell line (lane *5*) and PMA-treated (5 ng/ml) U937 cells (lane *6*) were included as controls. (*B*) $\beta 2$ mRNA levels in purified human tonsillar B cells either nonactivated (lane *1*) or activated with PMA (5 ng/ml) for 24, 48, or 72 h (lanes *2*, *3*, and *4*, respectively). 3-d PMA-activated (5 ng/ml) U937 mRNA was loaded in lane *5*. In every case, a similar amount of RNA (10 μg) was loaded, as controlled by ethidium bromide staining of the gel.

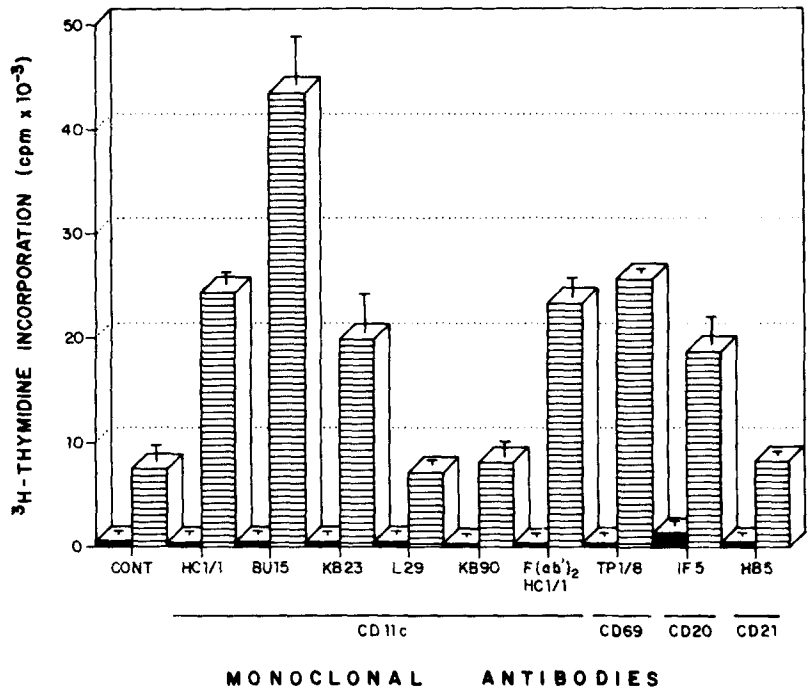

Figure 4. B cell proliferation induced by anti-CD11c, anti-CD20, anti-CD69, and anti-CD21 mAbs. mAbs were added at day 0 in the absence (*filled bars*) or presence (*hatched bars*) of PMA (3 ng/ml). The [^{3}H]TdR uptake was measured at day 3. HC1/1, BU15, L29, and F(ab')$_2$ fragments of HC1/1, 1F5, TP1/8, and HB5 mAbs were added at 2 μg/ml. Anti-CD11c KB23 and KB90 mAbs were added as a 1:600 ascites dilution.

linking of CD11c mAb did not seem to be necessary since either soluble or crosslinked HC1/1 mAb elicited similar proliferative responses (data not shown), in contrast with results obtained with other β2 integrin mAbs on T cell proliferation (12).

This integrin-mediated B cell proliferation was CD11c specific since CD11a TS1/11 and CD11b Bear 1 mAbs failed to alter B cell proliferation, although a wider panel of mAbs are currently been testing (see below). The CD18 TS1/18 mAb consistently induced small increases of [^{3}H]TdR incorporation ranging from 1- to 1.5-fold (data not shown).

Finally, to ascertain whether the continuous presence of a protein kinase C (PKC) activator was necessary for the triggering of the mitogenic responses through CD11c, B cells were activated with PDB for 2 d. After removal of the phorbol ester, the CD11c expression remained constant at days 3 and 4 (data not shown), and B cell proliferation could be triggered with CD11c mAb (Table 2). Therefore, the continuous

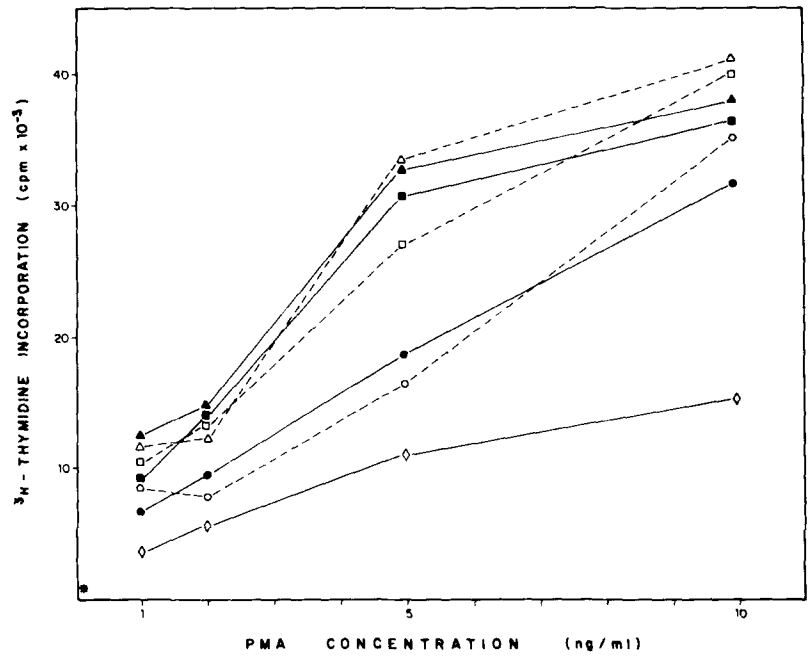

Figure 5. B cell proliferation induced by intact and F(ab')$_2$ fragments of anti-CD11c mAb. Purified tonsillar B cells were cultured with PMA with either intact (*solid lines*) or F(ab')$_2$ fragments (*dotted lines*) of HC1/1 mAb at different doses. (◇) No mAb; (○ and ●) 0.1 μg/ml; (□ and ■) 1 μg/ml; (△ and ▲) 5 μg/ml. *Cells cultured with complete medium alone.

Table 2. *B Cell Proliferation through CD11c in the Absence of Concomitant PKC Activators*

	Not washed*		Washed‡	
	− HC1/1	+ HC1/1	− HC1/1	+ HC1/1
Control	0.25 ± 0.15	0.12 ± 0.05	0.35 ± 0.21	0.28 ± 0.12
PDB	5.31 ± 0.91	14.35 ± 1.63	0.65 ± 0.18	7.82 ± 1.32
PMA	5.25 ± 1.25	17.25 ± 2.01	4.60 ± 1.35	12.32 ± 2.13

Purified tonsillar B cells were activated with PDB (10 ng/ml) or PMA (5 ng/ml). [^{3}H]TdR incorporation (cpm × 10^{-3}) was measured at day 4. [^{3}H]TdR uptake was calculated as mean of triplicate values. Data represent one experiment out of five different assays.

* At 48 h, 2 μg/ml of HC1/1 mAb was added.

‡ In the washed condition, after 2 d, cells were gently washed with RPMI 1640, resuspended in complete medium, and, upon overnight incubation, 2 μg/ml of HC1/1 mAb was added.

presence of a PKC activator is not required for the comitogenic signals delivered through CD11c.

B Cell Activation Induces CD11c-mediated Cell Attachment to Fibrinogen. Fibrinogen Reverts B Cell Proliferation Triggered by CD11c mAb Very recently, it has been reported that activated neutrophils interact with fibrinogen (FG) through both Mac-1 (42, 43) and p150,95 (44). To analyze the functional adhesive capacities of CD11c/CD18 in the absence of CD11b expression, FG binding assays were performed on either resting and activated B cells. As shown in Fig. 6, PMA-treated B cells, but not nonactivated B cells, were able to interact with FG. The specific involvement of CD11c/CD18 in the B cell attachment to FG was demonstrated by the ability of CD11c and CD18 mAbs to block the binding (Fig. 6). Adhesiveness to collagen (COL) and to a fibronectin fragment (FN38) containing the domain recognized by VLA-4 (CD49d/CD29) integrin was included as control (32). No inhibitory effects of CD11c HC1/1 and CD18 TS1/18 mAbs in B cell attachment to FN38 were observed. Likewise, anti-CD49d (VLA-α4) HP2/1 mAb did not inhibit B cell binding to FG (data not shown). Therefore, CD11c/CD18 integrin acts as a receptor for FG on human B cells in the absence of CD11b surface expression.

A perfect correlation was observed between the comitogenic activity of CD11c mAbs and their inhibitory effect on B cell attachment to FG: HC1/1, KB23, and BU15 were able to trigger B cell proliferation and to inhibit the cell binding to FG. This fact led us to explore whether FG had any effect, either mitogenic or inhibitory, on the CD11c mAb–mediated B cell proliferation. As shown in Table 3, FG inhibited the proliferation induced by CD11c mAbs. The partial inhibitory effect of FG probably reflects the inaccessibility of CD11c to the added mAbs caused by the redistribution of the antigen induced by the surface-bound ligand, a fact already observed for Mac-1 (45).

Discussion

The results reported here reveal that CD11c/CD18 can be induced on purified human B cells upon activation with several stimuli such as phorbol esters or either SAC or anti-μ antibodies combined with cytokines. In addition, the CD11c/CD18 integrin is involved in both B cell activation and adhesion, since mAbs against this molecule trigger B cell proliferation and block cell binding to FG.

The coexpression of CD11b and CD11c is known to be restricted to granulocytes, monocytes, and NK cells (16, 17). CD11c expression has been also demonstrated on dendritic cells (18, 19) and on some transformed T lymphocytes (20). Among the B cell lineage, the expression of CD11c has been reported in the HCL cells and in some B-CLL (21, 22, 46), which are also CD11b$^+$ and CD5$^+$ (22). Recently, a small CD5$^+$ CD11c$^+$ B cell subset on peripheral blood from normal donors has been detected and proposed as the normal counterpart of B-CLL cells (46).

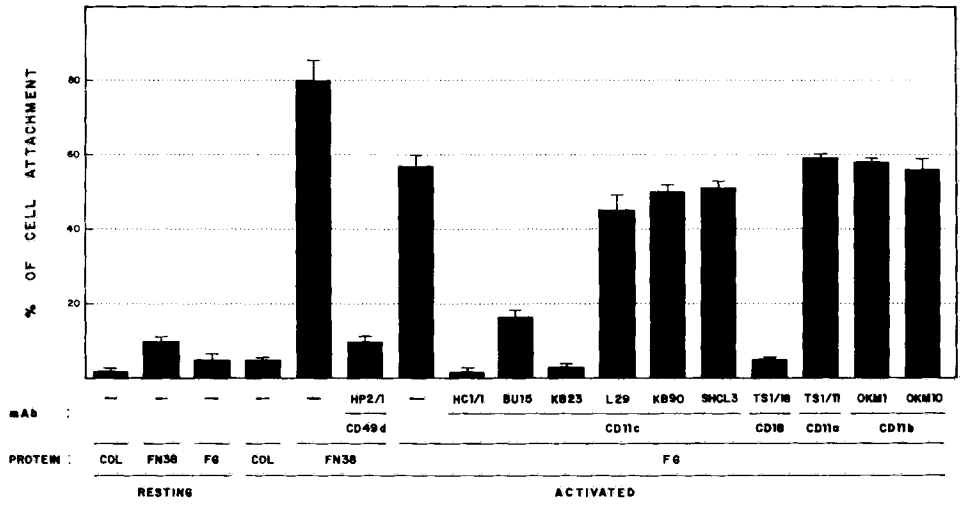

Figure 6. Binding of either nonactivated or 3-d activated tonsillar B cells to plates coated with type I collagen, a 38-kD proteolytic fragment of fibronectin (FN38), or FG. B cells were activated with PMA (5 ng/ml) and adhesion assays were performed as described in Materials and Methods. mAb concentrations: HC1/1, BU15, L29, TS1/18, and TS1/11 were used at 5 μg/ml; HP2/1 was used as culture supernatant at a 25% final volume concentration; KB23, KB90, OKM1, and OKM10 as a 1/400 ascites dilution; and S HCL-3 at a 1/200 ascites dilution. The figure represents the average of five different experiments.

Table 3. *Fibrinogen Blocks the CD11c mAb-mediated B Cell Proliferation*

	−FG	+FG
Medium	0.67 ± 0.32	0.71 ± 0.34
HC1/1 mAb	0.55 ± 0.24	0.68 ± 0.18
PMA	7.78 ± 0.87	6.88 ± 1.13
PMA + HC1/1 mAb	26.47 ± 1.81	14.45 ± 1.92

Effect of FG in CD11c mAb-mediated B cell proliferation. FG was coated to plates at 20 μg/ml (100 μl/well) overnight at 4°C. Plates were gently washed and tonsillar B cells were added and activated with PMA (2 ng/ml) and/or HC1/1 mAb (5 μg/ml). Each condition was performed by triplicate. [^{3}H]TdR uptake was measured at day 3. These data represent one experiment out of four different assays.

The expression of CD11c by leukemic B cells suggested that its expression could be associated with cell activation processes. This fact has been confirmed by our finding of induction of CD11c expression during B cell activation. It is worth noting the lack of induction of CD11b, as opposed to its expression by B-CLL cells, suggesting a different regulation in the expression of both CD11b and CD11c, on normal and transformed human B cells.

After activation, B cells undergo a number of phenotypic changes in the expression of their surface molecules that are closely related to the activation signal (23–32). For example, only phorbol esters induce CD5 expression on B cells, whereas other stimuli such as anti-μ Ab and several cytokines fail to induce this B cell activation antigen (29). Our data demonstrate that CD11c expression is mainly induced after phorbol ester treatment, although it could also be obtained upon treatment with the more physiological B cell activation agents SAC or anti-μ Ab in combination with cytokines. The slow pattern of induction of CD11c on B cells resembles that observed on U937 and HL60 myelomonocytic cell lines after PMA treatment (47) and may reflect the existence of phorbol ester responsive elements on the promoter region of the CD11c gene (A.L. Corbi, unpublished observations).

Although originally integrins were believed to participate only in adhesion processes, recent reports demonstrate that they are also involved in cell activation. Thus, comitogenic signals can be triggered through VLA-4, VLA-5, and VLA-6 on activated T cells (6–9), and the expression of certain matrix-degrading protease genes (48) and transcription factors (49) is induced through VLA-5. Moreover, other nonintegrin adhesion molecules such as CD44 homing receptor and the CD2/LFA-3 pair have been also implicated in cell activation (50–53). Our data constitutes the first evidence about the implication of integrins in B cell proliferation and further strengthens the link between adhesion and cell activation. Other β2 integrins have been also implicated in cell activation. Thus, CD11b mAbs have been reported to induce macrophage activation (54), and both CD11a and CD18 subunits are also involved in T cell activation (10–15). On B cells, LFA-1 mAbs have been reported to inhibit T cell–dependent B cell proliferation and differentiation, although CD11a mAbs have no effect on purified B cells (55–58). These data correlate with our results about the inefficiency of the anti-CD11a TS1/11 mAbs to alter the proliferative response induced by PMA. The lack of effect of LFA-1 mAbs on B cell proliferation as compared to that observed for T cells could reflect a different role of this integrin in T and B cell activation processes. At present, we are analyzing a wider panel of CD11a mAbs to further clarify the LFA-1 involvement on B cell activation.

The comitogenic effect of CD11c mAbs does not seem to be mediated via PKC activation. Moreover, none of the mitogenic anti-CD11c mAbs induced any change in the intracellular Ca^{2+} levels of activated $CD11c^+$ B cells, even when a crosslinking agent was added (A.A. Postigo, unpublished observations). Furthermore, the mitogenic response elicited by CD11c mAbs did not require the continuous presence of PKC activators, suggesting that CD11c cell surface expression is enough to allow B cell proliferation via CD11c. To the best of our knowledge, no data exist about the involvement of CD11c/CD18 on cell proliferation, and further research is required to ascertain whether this activation effect could also be observed on other CD11c-expressing cell types. The difference in the proliferative response elicited by the CD11c mAbs assayed here suggests the existence of different functional domains on CD11c. It would be of interest to correlate these functional domains with those involved in the binding of CD11c/CD18 to its endothelial ligand (59).

Among the β2 integrins, the functional role of CD11c is the least known, and some controversy exists about its ligand-binding abilities. Thus, while some studies have implicated CD11c/CD18 in the cytotoxicity mediated by certain T cell clones (20), others have questioned this involvement (17). iC3b has also been postulated to be a ligand for CD11c (60) although CD11c/CD18-transfected COS cells fail to bind to this complement factor (3). CD11c/CD18 has also been involved in granulocyte binding to endothelium, although playing only an ancillary role in respect to that of LFA-1 and Mac-1 (61, 62), and contradictory evidence about the participation of CD11c/CD18 in monocyte adhesion to endothelium has been reported (63, 64). Our results indicate that CD11c/CD18 is a receptor for FG on B cells, a fact that has been recently reported on TNF-activated neutrophils (44). So far, CD11c/CD18 is the only β2 integrin without an identified endothelial ligand, although the existence of such as ligand has been demonstrated with purified CD11c protein (59). The other two β2 integrins, LFA-1 and Mac-1, share intercellular adhesion molecule 1 (ICAM-1) as a counter-receptor on endothelium, through recognition of different domains of the molecule (65, 66). The FG-binding ability of Mac-1 and p150,95 follows the same scheme: both are able to interact with the same ligand, although recognizing distinct domains (42, 44). Given the structural similarity among them, it is tempting to speculate that the three leukocyte integrins can recognize a common set of ligands.

The ability of the mitogenic CD11c mAbs to block B cell binding to FG suggested that FG could be involved in B cell activation. However, FG did not trigger mitogenic responses

although it was able to inhibit CD11c mAb–mediated B cell proliferation. This fact could be explained because FG may bind to a CD11c region close, but not identical, to that defined by the mitogenic CD11c mAbs.

In summary, our data constitute the first evidence for the induction of CD11c/CD18 on nontransformed B cells and its involvement on cell activation. The functional capability of certain CD11c mAbs to trigger B cell proliferation and to inhibit FG binding indicate that this integrin plays a role in the regulation of both B cell activation and adhesion processes. mAbs directed to CD11c could exert their mitogenic effects by mimicking the interaction of CD11c with its natural ligand. Whether iC3b or the putative endothelial ligand are able to induce proliferative responses on $CD11c^+$ B cells remains to be explored. To that effect, purified endothelial ICAM-1 molecule is able to trigger mitogenic responses by binding to LFA-1 on T cells (15), and the proliferative effects of other complement factors on normal B lymphocytes and B cell lines have been also documented (67–71). The triggering of B cell proliferative responses through CD11c/CD18 by iC3b and endothelium is currently under study in our laboratory.

We thank Drs. Clark, Johnson, Lanier, Pulford, and Schwarting for the kind supply of mAbs. We also thank D. Hernández and M. Vitón for their technical assistance in flow cytometry.

This work was supported by grants from INSALUD (FIS 089/0067 and 090/0113) and from Fundación Ramón Areces.

Address correspondence to Antonio A. Postigo, Servicio de Inmunología, Hospital de la Princesa, C/Diego de León, 62, 28006 Madrid, Spain.

Received for publication 13 June 1991 and in revised form 2 August 1991.

References

1. Springer, T.A. 1990. Adhesion receptors in the immune system. *Nature (Lond.).* 346:425.
2. Ruoslahti, E. 1991. Integrins. *J. Clin. Invest.* 87:1.
3. Larson, R.S., and T.A. Springer. 1990. Structure and function of leukocyte integrins. *Immunol. Rev.* 114:180.
4. Hemler, M.E. 1990. VLA proteins in the integrin family: structures, function, and their role in leukocytes. *Annu. Rev. Immunol.* 8:365.
5. Plow, E.F., and M.H. Ginsberg. 1989. Cellular adhesion: GpIIb-IIIa as a prototypic adhesion receptor. *Prog. Hemostasis Thromb.* 9:117.
6. Matsuyama, T., A. Yamada, J. Kay, K.M. Yamada, S.K. Akiyama, S.F. Schlossman, and C. Morimoto. 1989. Activation of CD4 cells by fibronectin and anti-CD3 antibody. A synergistic effect mediated by the VLA-5 fibronectin receptor complex. *J. Exp. Med.* 170:1133.
7. Shimizu, Y., G.A. van Seventer, K.J. Horgan, and S. Shaw. 1990. Costimulation of proliferative responses of resting $CD4^+$ T cell by the interaction of VLA-4 and VLA-5 with fibronectin or VLA-6 with laminin. *J. Immunol.* 145:59.
8. Davis, L.S., N. Oppenheimer-Marks, J. Bednarczyk, B.W. McIntyre, and P.E. Lipsky. 1990. Fibronectin promotes proliferation of naive and memory T cells by signaling through both the VLA-4 and VLA-5 integrin molecules. *J. Immunol.* 145:785.
9. Yamada, Y., Y. Nojima, K. Sugita, N.H. Dang, S.F. Schlossman, and C. Morimoto. 1991. Cross-linking of VLA/CD29 molecule has a co-mitogenic effect with anti-CD3 on CD4 cell activation in serum-free culture system. *Eur. J. Immunol.* 21:319.
10. Dongworth, D.W., F.M. Gotch, J.E. Hildreth, A. Morris, and A.J. McMichael. 1985. Effects of monoclonal antibodies to the alpha and beta chains of the human lymphocytes function-associated (H-LFA-1) antigen on T lymphocyte functions. *Eur. J. Immunol.* 15:888.
11. Carrera, A.C., M. Rincón, F. Sánchez-Madrid, M. López-Botet, and M.O. de Landázuri. 1988. Triggering of co-mitogenic signals in T cell proliferation by anti-LFA-1 (CD18, CD11a), LFA-3 and CD7 monoclonal antibodies. *J. Immunol.* 141:1919.
12. van Noesel, C., E. Miedema, M. Brouwer, M.A. de Rie, L.A. Aarden, and R.A.W. Lier. 1988. Regulatory properties of LFA-1 α and β chains in human T-lymphocyte activation. *Nature (Lond.).* 333:850.
13. Geppert, T.D., and P.E. Lipsky. 1988. Activation of T lymphocytes by immobilized monoclonal antibodies to CD3. Regulatory influences of monoclonal antibodies to additional T cell determinants. *J. Clin. Invest.* 81:1497.
14. Wacholtz, M.C., S.S. Patel, and P.E. Lipsky. 1989. Leukocyte function-associated antigen 1 is an activation molecule for human T cells. *J. Exp. Med.* 170:431.
15. van Seventer, G.A., Y. Shimizu, K.J. Horgan, and S. Shaw. 1990. The LFA-1 ligand ICAM-1 provides an important costimulatory signal for T cell receptor-mediated activation of resting T cells. *J. Immunol.* 144:4579.
16. Hogg, N., L. Takacs, D.G. Palmer, Y. Salverdran, and C. Allen. 1986. The p150,95 is a marker of human phagocytes: comparison with expression of class II molecules. *Eur. J. Immunol.* 16:240.
17. Lanier, L.L., M.A. Arnaout, R. Schwarting, N.L. Warner, and G.D. Ross. 1985. p150,95: third member of the LFA-1/CR3 polypeptide family identified by anti-Leu M5 monoclonal antibody. *Eur. J. Immunol.* 15:713.
18. Metlay, J.P., M.D. Witmer-Pack, R. Agger, M.T. Crowley, DeS. Lawles, and R.M. Steinman. 1990. The distinct leukocyte integrins of mouse spleen dendritic cells as identified with

new hamster monoclonal antibodies. *J. Exp. Med.* 171:1753.
19. Freundenthal, P.S., and R.M. Steinman. 1990. The distinct surface of human blood dendritic cells, as observed after an improved isolation method. *Proc. Natl. Acad. Sci. USA.* 87:7698.
20. Keizer, G.F., J. Borst, W. Kisser, R. Schwarting, J.E. de Vries, and C.G. Figdor. 1987. Membrane glycoprotein p150,95 of human cytotoxic T cell clones is involved in conjugate formation with target cells. *J. Immunol.* 138:3130.
21. Schwarting, R., H. Stein, and C.Y. Wang. 1985. The monoclonal antibodies α S-HCL 1 (αLeu-14) and α S-HCL 3 (αLeu-M5) allow the diagnosis of hairy cell leukemia. *Blood.* 65:974.
22. De la Hera, A., M. Alvarez-Mon, F. Sánchez-Madrid, C. Martinez-A, and A. Durantez. 1988. Co-expression of Mac-1 and p150,95 on CD5+ B cells. Structural and functional characterization in human chronic lymphocytic leukemia. *Eur. J. Immunol.* 18:1131.
23. Boyd, A.W., K.C. Anderson, A.S. Freedman, D.C. Fisher, B. Slaughenhoupt, S.F. Schlossman, and L.M. Nadler. 1985. Studies of in vitro activation and differentiation of human B lymphocytes. I. Phenotypic and functional characterization of the B cell population responding to anti-Ig antibody. *J. Immunol.* 134:1516.
24. Aman, P., J. Gordon, and G. Klein. 1984. TPA (12-0-tetradecanoyl phorbol 13 acetate) activation and differentiation of human peripheral B lymphocytes. *Immunology.* 51:27.
25. Freedman, A.S., A.W. Boyd, A. Berribi, J.C. Horowitz, D.N. Levy, K.J. Rosen, J. Daley, B. Slaughenhoupt, H. Levine, and L.M. Nadler. 1987. Expression of B cell activation antigens on normal and malignant B cells. *Leukemia (Baltimore).* 1:9.
26. DeFrance, T., J.P. Aubry, F. Rousset, B. Vanberuliet, J.Y. Bonnefoy, N. Arai, Y. Takebe, T. Yokota, F. Lee, K. Arai, J. de Vries, and J. Banchereau. 1987. Human recombinant interleukin 4 induces Fcε receptors (CD23) on normal human B lymphocytes. *J. Exp. Med.* 165:1459.
27. Sánchez-Mateos, P., M. Cebrián, A. Acevedo, M. López-Botet, M.O. de Landázuri, and F. Sánchez-Madrid. 1989. Expression of GP33/27,000 MW activation inducer molecule (AIM) on human lymphoid tissues. Induction of cell proliferation on thymocyte and B lymphocyte by anti-AIM antibodies. *Immunology.* 68:72.
28. Miller, R.A., and J. Gralow. 1984. The induction of Leu-1 antigen expression in human malignant and normal B cells by phorbol myristic acetate (PMA). *J. Immunol.* 133:3408.
29. Freedman, A.S., G. Freeman, J. Whitman, J. Segil, J. Daley, H. Levine, and L.M. Nadler. 1989. Expression and regulation of CD5 on in vitro activated human B cells. *Eur. J. Immunol.* 19:849.
30. Freedman, A.S., A.W. Boyd, K.C. Anderson, D.C. Fisher, S.F. Schlossman, and L.M. Nadler. 1985. B5, a new B cell restricted activation antigen. *J. Immunol.* 134:2228.
31. Yokochi, T., R.D. Holly, and E.A. Clark. 1982. B lymphoblast antigen (BB1) expressed on Epstein-Barr virus-activated B cell blasts, B lymphoblastoid cell lines, and Burkitt's lymphomas. *J. Immunol.* 128:823.
32. Postigo, A.A., R. Pulido, M.R. Campanero, A. Acevedo, A. García-Pardo, A.L. Corbí, F. Sánchez-Madrid, and M.O. de Landázuri. 1991. Differential expression of VLA-4 integrin by resident and peripheral blood B lymphocytes. Acquisition of functional active α4β1 fibronectin receptors upon cell activation. *Eur. J. Immunol.* In press.
33. Cabañas, C., F. Sánchez-Madrid, A. Acevedo, T. Bellón, J.M. Fernandez, V. Larraga, and C. Bernabeu. 1988. Characterization of a CD11c-reactive monoclonal antibody (HC1/1) obtained by immunizing with phorbol ester differentiated U937 cells. *Hybridoma.* 7:167.
34. Sánchez-Madrid, F., J. Nagy, E. Robbins, P. Simon, and T.A. Springer. 1983. A human leukocyte differentiation antigen family with distinct alpha subunits and a common beta subunit: the lymphocytes function-associated antigen (LFA-1), the C3Rbi complement receptor (OKM1/Mac-1), and the p150,95 molecule. *J. Exp. Med.* 158:1785.
35. Keizer, G.D., J. Borst, C.G. Figdor, H. Spits, F. Miedema, C. Terhorst, and J.E. de Vries. 1985. Biochemical and functional characteristics of the human leukocyte membrane antigen family LFA-1, Mol, and p150,95. *Eur. J. Immunol.* 15:1142.
36. Cebrián, M., E. Yagüe, M. Rincón, M. López-Botet, M.O. de Landázuri, and F. Sánchez-Madrid. 1988. Triggering of T cell proliferation through AIM, and activation inducer molecule, expressed on activated human lymphocytes. *J. Exp. Med.* 168:1621.
37. Bernabeu, C., A.C. Carrera, M.O. de Landázuri, and F. Sánchez-Madrid. 1987. Interaction between the CD45 antigen and phytohemagglutinin. Inhibitory effect of the lectin-induced T cell proliferation by anti-CD45 monoclonal antibody. *Eur. J. Immunol.* 17:1461.
38. Sánchez-Madrid, F., M.O. de Landázuri, G. Morago, M. Cebrián, A. Acevedo, and C. Bernabeu. 1986. VLA-3: A novel polypeptide association within the VLA molecular complex: cell distribution and biochemical characterization. *Eur. J. Immunol.* 16:1343.
39. Parham, P., H.J. Androlewick, F.M. Brodsky, N.I. Holmes, and J.P. Ways. 1982. Monoclonal antibodies: purification, fragmentation, and application to structural and functional studies of class I MHC antigens. *J. Immunol. Methods.* 53:133.
40. Corbi, A.L., L.J. Miller, K. O'Connor, R.S. Larson, and T.A. Springer. 1987. cDNA cloning and complete primary structure of the α subunit of a leukocyte glycoprotein p150,95. *EMBO (Eur. Mol. Biol. Organ.) J.* 6:4023.
41. Kishimoto, T.K., K. O'Connor, A. Lee, T.M. Roberts, and T.A. Springer. 1987. Cloning of the β subunit of the leukocyte adhesion proteins: homology to an extracellular matrix receptor defines a novel supergene family. *Cell.* 48:681.
42. Wright, S.D., J.I. Weitz, A.J. Huang, S.M. Levin, S.C. Silverstein, and J.D. Loike. 1988. Complement receptor type three (CD11b/CD18) of human polymorphonuclear leukocytes recognizes fibrinogen. *Proc. Natl. Acad. Sci. USA.* 85:7734.
43. Altieri, D.C., R. Bader, P.M. Manucci, and T.S. Edgington. 1988. Specificity of the cellular adhesion receptor MAC-1 encompasses on inducible recognition specificity for fibrinogen. *J. Cell Biol.* 107:1893.
44. Loike, J.D., B. Sodeik, L. Cao, S. Leucona, J.I. Weitz, P.A. Detmers, S.D. Wright, and S.C. Silverstein. 1991. CD11c/CD18 on neutrophils recognizes a domain at the N terminus of the Aα chains of fibrinogen. *Proc. Natl. Acad. Sci. USA.* 88:1044.
45. Wright, S.D., P.A. Reddy, M.T.C. Jong, and B.W. Erickson. 1987. C3bi receptor (complement receptor type 3) recognizes a region of complement protein C3 containing the sequence Arg-Gly-Asp. *Proc. Natl. Acad. Sci. USA.* 84:1965.
46. Wormsley, S.B., S.M. Baird, N. Gadol, K.R. Rai, and R.E. Sobol. 1990. Characteristics of $CD11c^+$ $CD5^+$ chronic B-cell leukemias and the identification of novel peripheral blood B-cell subsets with chronic lymphoid leukemia immunophenotypes. *Blood.* 76:123.
47. Miller, L.J., R. Schwarting, and T.A. Springer. 1986. Regulated expression of the Mac-1, LFA-1 and p150,95 glycoprotein family during leukocyte differentiation. *J. Immunol.*

137:2891.
48. Werb, Z., P.M. Tremble, O. Behrendtsen, E. Crowley, and C.M. Damsky. 1989. Signal transduction through the fibronectin receptor induces collagenase and stromelysin gene expression. *J. Cell Biol.* 109:877.
49. Yamada, A., T. Nikaido, Y. Nojima, S.F. Schlossman, and C. Morimoto. 1991. Activation of human CD4 T lymphocytes. Interaction of fibronectin with VLA-5 receptor on CD4 cells induces the AP-1 transcription factor. *J. Immunol.* 146:53.
50. De la Hera, A., A. Acevedo, W. Marston, and F. Sánchez-Madrid. 1989. Function of CD44 (Pgp-1) homing receptor in human T cell precursors. *Int. Immunol.* 1:598.
51. Huet, S., H. Groux, B. Caillou, H. Valentin, A.H. Prieur, and A. Bernard. 1989. CD44 contributes to T cell activation. *J. Immunol.* 143:798.
52. Shimizu, Y., G.A. van Seventer, R. Siraganian, L. Wahl, and S. Shaw. 1989. Dual role of the CD44 molecule in T cell adhesion and activation. *J. Immunol.* 143:2457.
53. Meuer, S.C., R.E. Hussey, M. Fabbi, D. Fox, O. Acuto, K.A. Fitzgerald, J.C. Hodgdon, J.P. Protentis, S.F. Schlossman, and E.L. Reinherz. 1984. An alternative pathway of T cell activation: a functional role of the 50 KDa T11 sheep erythrocyte receptor protein. *Cell.* 36:897.
54. Ding, A., S.D. Wright, and C. Nathan. 1987. Activation of mouse peritoneal macrophages by monoclonal antibodies to Mac-1 (complement receptor type 3). *J. Exp. Med.* 165:733.
55. Fischer, A., A. Durandy, G. Sterkers, and C. Griscelli. 1986. Role of the LFA-1 molecule in cellular interactions required for antibody production in humans. *J. Immunol.* 136:3198.
56. Howard, D.R., A.C. Eaves, and F. Takei. 1986. Lymphocyte function-associated antigen (LFA-1) is involved in B cell activation. *J. Immunol.* 136:4013.
57. Tohma, S., S. Hirohata, and P.E. Lipsky. 1991. The role of CD11a/CD18-CD54 interactions in human T cell-dependent B cell activation. *J. Immunol.* 146:492.
58. Torimoto, Y., K. Sugita, D.S. Weinberg, N.H. Dang, C. Donahue, N.L. Letvin, S.F. Schlossman, and C. Morimoto. 1991. Development of a monoclonal antibody, anti-6C2, which is involved in the interaction of CD4 T helper cells and activated B cells. *J. Immunol.* 146:2176.
59. Stacker, S.A., and T.A. Springer. 1991. Leukocyte integrin p150,95 (CD11c/CD18) functions as an adhesion molecule binding to a counter-receptor on stimulated endothelium. *J. Immunol.* 146:648.
60. Micklem, K.J., and R.B. Sim. 1985. Isolation of complement-fragment: C3b- binding proteins by affinity chromatography. The identification of p150,95 as an iC3b-binding protein. *Biochem. J.* 231:233.
61. Harlan, J.M., P.D. Killer, F.M. Senecal, B.R. Schwartz, E.K. Yee, R.F. Taylor, P.G. Beatty, T.H. Price, and M.D. Ochs. 1987. The role of neutrophil membrane glycoprotein gp150 in neutrophil membrane adherence to endothelium in vitro. *Blood.* 66:167.
62. Luscinskas, F.W., A.F. Bruck, M.A. Arnaout, and M.A. Gimbrone. 1989. Endothelial adhesion molecule-1-dependent and leukocyte (CD11/CD18)-dependent mechanisms contribute to polymorphonuclear leukocyte adhesion to cytokine-activated human vascular endothelium. *J. Immunol.* 142:2257.
63. te Velde, A.A., G.D. Keizer, and C.G. Figdor. 1987. Differential function of LFA-1 family molecules (CD11 and CD18) in adhesion of human monocytes to melanoma and endothelial cells. *Immunology.* 61:261.
64. Beekhuizen, M., A.J. Corsèl-van Tilburg, and R. van Furth. 1990. Characterization of monocyte adherence to human macrovascular and microvascular endothelial cells. *J. Immunol.* 145:510.
65. Marlin, S.D., and T.A. Springer. 1987. Purified intercellular adhesion molecule (ICAM-1) is a ligand for lymphocyte function-associated antigen 1 (LFA-1). *Cell.* 51:813.
66. Diamond, M.S., D.E. Staunton, A.R. de Fougerolles, S.A. Stacker, J. García-Aguilar, M.L. Hibbs, and T.A. Springer. 1990. ICAM-1 (CD54): A counter receptor for Mac-1 (CD11b/CD18). *J. Cell Biol.* 111:3129.
67. Nemerow, G.R., M.E. McNaughton, and N.R. Cooper. 1985. Binding of monoclonal antibody to the Epstein-Barr virus (EBV)/CR2 receptor induces activation and differentiation of human B lymphocytes. *J. Immunol.* 135:3068.
68. Bohnsack, J.F., and N.R. Cooper. 1988. CR2 ligands modulate human B cell activation. *J. Immunol.* 141:2569.
69. Tsokos, G.C., J.D. Lambris, F.D. Finkelman, E.D. Anastassiou, and C.H. June. 1990. Monovalent ligands of complement receptor 2 inhibit whereas polyvalent ligand enhance anti-Ig induced human B cell intracytoplasmic free calcium concentration. *J. Immunol.* 144:1640.
70. Hatzfeld, A., E. Fischer, J.P. Levesque, R. Perrin, J. Hatzfeld, and M.D. Kaztchkine. 1988. Binding of C3 and C3dg to the CR2 complement receptor induces growth of an Epstein-Barr virus positive human B cell line. *J. Immunol.* 140:170.
71. Thoman, M.L., J.L. Meuth, E.L. Morgan, W.O. Weigle, and T.E. Hugli. 1984. C3d-k, an kallikrein cleavage fragment of iC3b, is a potent inhibitor of cellular proliferation. *J. Immunol.* 133:2629.