Engaging CD19 or Target of an Antiproliferative Antibody 1 on Human B Lymphocytes Induces Binding of B Cells to the Interfollicular Stroma of Human Tonsils via Integrin α4/β1 and Fibronectin

By Susanne Behr and Folke Schriever

From the Department of Hematology and Oncology, Virchow University Hospital, Humboldt University Berlin, Berlin, Germany

Summary

Adhesion of B lymphocytes within the different compartments of secondary lymphoid organs is essential for the function of the humoral immune response. It is not currently known how the temporary immobilization of B cells in distinct areas of this complex microenvironment is regulated. The present study aimed at defining B cell antigens that initiate binding of B cells to human tonsil sections in situ. Engaging the B cell antigens CD19 and target of an antiproliferative antibody 1 (TAPA-1) with monoclonal antibodies induced adhesion of these B cells to the interfollicular stroma. This binding occurred through the integrin α4β1 on the B cell surface and via the extracellular matrix protein fibronectin expressed in the interfollicular compartment of the tonsil. Signaling through either antigen, CD19 or TAPA-1, depended on tyrosine kinases. Binding induced by engaging CD19 required an intact cytoskeleton, whereas TAPA-1–transmitted adhesion did not. We suggest that CD19 and TAPA-1 have a novel and unique function by regulating an α4β1/fibronectin-mediated binding of B cells to the interfollicular stroma of lymphoid tissues.

Adhesion of human B lymphocytes within the microenvironment of secondary lymphoid organs is essential for the function of the humoral immune response (1, 2). B lymphocytes migrate through secondary lymphoid organs by binding with their cell-surface adhesion molecules to constitutively expressed ligands within the different tissue compartments (1, 2). Guided by these "traffic signals" (1), B cells adhere to and emigrate through the vascular endothelium and traverse the interfollicular stroma, where they make contact with antigen-presenting dendritic cells (3, 4), T cells, and stromal cells. Activated by signals provided in this cellular network (5), B cells migrate into the lymphoid follicle, where they undergo antigen-driven selection by apoptosis (6). In this complex network built by follicular dendritic cells (FDCs)[1] (7–9), T cells, and other germinal center B cells, B cells receive a multitude of signals that determine whether these cells differentiate into memory cells or plasma cells or whether they are eliminated by apoptosis (10). Analogous to in vitro–observed adhesion via β1 (11–17) and β2 integrins (18, 19), specificity of B cell binding within these different compartments of the lymphoid tissue is presumably influenced by transient activation of adhesion molecules (20). It is currently unknown, however, what determines the temporary immobilization of B cells in the different areas of lymphoid tissues.

Therefore, we sought to identify B cell antigens that initiate binding of B cells to distinct areas of the lymphoid tissue. CD19 and target of an antiproliferative antibody 1 (TAPA-1, CD81), which, together with CD21 and Leu-13 (21), form a B cell membrane complex (22–24), were found to have the unique function of inducing an integrin α4β1(very late antigen 4, CD49d/CD29)–dependent adhesion of B cells to the interfollicular stroma. Engaging CD19 or TAPA-1 with mAbs initiated a specific and tyrosine kinase–dependent binding of B cells to fibronectin (FN). CD19 and TAPA-1 thus appear to have the novel function of directing an α4β1/FN-mediated immobilization of B cells in the interfollicular matrix of secondary lymphoid organs.

Materials and Methods

Antibodies and Reagents. The following antibodies were used: 5A6 (25) (anti–TAPA-1, generous gift of S. Levy, Stanford University, Stanford, CA), anti-α4β7 (26) (generously donated by A.

[1] *Abbreviations used in this paper:* ECM, extracellular matrix; FDC, follicular dendritic cell; FN, fibronectin; ICAM, intercellular adhesion molecule; LFA, lymphocyte function–associated antigen; TAPA, target of an antiproliferative antibody; VCAM, vascular cell adhesion molecule.

 J. Exp. Med. © The Rockefeller University Press • 0022-1007/95/11/1191/09 $2.00
Volume 182 November 1995 1191–1199

Lazarovits, John P. Robarts Research Institute, University Hospital and the University of Western Ontario, Ontario, Canada), and 4B9 (27) (anti–vascular cell adhesion molecule [VCAM] 1, kindly given by T. M. Carlos, Division of Hematology, Harborview Medical Center, Seattle, WA). Anti-CD19 (B43, BU12, HD237, HD37), anti-CD21 (BU36, BU42), anti-CD40 (G285, HD28), HI29 anti-CD53, and anti-CD102 (intercellular adhesion molecule [ICAM] 2) were obtained from the Fifth International Workshop on Human Leukocyte Differentiation Antigens. Rabbit anti-FN antiserum and rabbit antilamillin antiserum were from Sigma Chemical Company (St. Louis, MO). mAbs against the following antigens were obtained from Dianova-Immunotech (Hamburg, Germany): human IgM, CD10 (clone ALB 1), CD11a (25.3.1), CD18 (BL5), CD19 (J4.119), CD20 (BB6), CD21 (BL13), CD22 (SJ.10.1H11), CD23 (9P.25), CD29 (K20), CD32 (2E1), CD37 (BL14), CD40 (MAB89), CD44 (J173), CD45 RA (ALB11), CD46 (J4-48), CD49d (HP2/1), CD49e (SAM1), CD54 (84H10), CD58 (AICD58), CD70 (HNE 51), CD72 (J3.109), CD81 (JS64), and FN (120-5). All mAbs were purified and did not contain sodium azide. Staurosporine was obtained from Boehringer Mannheim (Mannheim, Germany). Genestein and herbimycin were purchased from Calbiochem Corp. (La Jolla, CA). Cycloheximide, cytochalasin B, and FN (bovine serum) were obtained from Sigma Chemical Co.

Cells. The pre–B cell lines Reh and Nalm-6 were given by M. Mapara and B. Dörken (Max Delbrück Zentrum, Berlin, Germany), and the Burkitt lymphoma cell lines Raji, Ramos, and Daudi were a gift of M. Hummel (Institute of Pathology, Benjamin Franklin University Hospital, Berlin, Germany) and of C. Schmidt (Virchow University Hospital, Berlin, Germany). All cell lines were maintained at 5% CO_2 in RPMI 1640 medium supplemented with 10% FCS, 1% glutamine, 1% penicillin, and 1% streptomycin. B cells were freshly isolated from human tonsils and were purified by centrifugation over a Ficoll gradient and subsequent negative selection using anti-CD3–coated magnetic beads (Dynabeads Dynal, Oslo, Norway) according to the instructions of the manufacturer. As shown by flow cytometry, these cell preparations consisted of >94% pure B cells.

Frozen Section Binding Assay. To study B cell adhesion to tonsils in situ, an in vitro frozen section binding assay (20, 28, 29) was performed with modifications. Normal human tonsils, obtained from routine elective tonsillectomies, were snap frozen, cut, placed on microscope slides, and dried overnight. B cells were applied at 8×10^6 cells/ml in 300 μl culture medium (containing 2.4 mM $MgCl_2$) on tissue sections within a 2-cm diameter circle marked with a water-repellent "Pap-pen" (Dako Corp., Carpinteria, CA) and were incubated under rotation (70 revolutions per min) for 20 min at room temperature. Thereafter, slides were decanted, were fixed for 4–6 h in cold 3% glutaraldehyde/PBS, and were counterstained with hemalaun. Bound cells were identified by phase contrast and light microscopy (×25). Induction of tissue binding of B cells by mAbs was assessed by incubating cells with purified mAbs (10 μg/ml) in 10% FCS in RPMI 1640 for 30 min at 4°C with gentle rotation. To investigate whether mAbs had a dose-dependent effect on adhesion, B cells were also treated with different concentrations of mAbs (5, 1, and 0.1 μg/ml). After they were washed three times, the cells were resuspended in medium and were applied to the tissue sections. To examine mAbs for their ability to block mAb-induced tissue binding of B cells, antibody-treated cells were incubated with a second set of mAbs and were examined for reduced tissue adherence. Reduction of binding was compared with adhesion of mAb-treated cells labeled with isotype-matched control mAbs and was qualitatively scored as complete (no cells bound) or incomplete inhibition (significantly fewer cells bound). Induction or inhibition of binding by mAbs was determined in at least three separate experiments using as control mAbs isotype-matched and B cell–reactive mAbs.

Assays of Cell Binding to Immobilized Fibronectin. Antibody-induced binding of B cells to purified FN was determined using plastic-immobilized FN. 24-well plastic plates (Falcon Labware, Oxnard, CA) were coated with 10 μg/ml FN in PBS for 3 h at 37°C and were washed extensively with PBS. B cells were treated with mAbs, were washed, and were incubated in triplicates at 1×10^5 cells per well for 20 min at 37°C. Unbound cells were removed by three washes with PBS, and adhering cells were fixed in 3% glutaraldehyde/PBS for 10 min at 4°C. The number of bound cells per well was determined as the mean number of cells counted in five representative high power fields (×400).

To determine FN-specific binding of mAb-treated cells, FN-coated plates were treated with anti-FN serum (dilution 1:500) or with control serum (antilamillin serum, dilution 1:500) for 1 h at room temperature. Plates were washed extensively before adding the cells. The reduction of binding to plates treated with anti-FN was determined in comparison with binding to FN-coated plates incubated with control serum. To test for an integrin α4-mediated adhesion to FN, mAb-treated cells were labeled with anti-α4 mAb and were applied to the plates. Reduction of binding to FN by anti-α4 mAb was determined relative to adhesion results obtained with control mAb (anti-CD20).

The involvement of intracellular pathways in mAb-induced binding of cells to FN was determined with a panel of different inhibitors. 8×10^6/ml cells were incubated for 30 min at 37°C with 5% CO_2 in media containing 10^2 nM staurosporine, 50 μM H7, 50 mg/ml genestein, 10 μg/ml cycloheximide, 1 μg/ml herbimycin, or 0.1 mM cytochalasin B, or were treated with control media. Control media had the same concentrations of DMSO solvent as were used in media containing the inhibitors. Cells were subsequently treated as described above with mAbs and were examined for adhesion to FN-coated plates. Reduction of binding due to inhibitors was determined relative to adhesion of mAb-treated cells incubated with control media.

Immunostaining. Immunophenotyping of B cells was performed by incubating cells with 10 μg/ml of each mAb for 30 min at 4°C. After washing, cells were labeled with fluoresceinated rabbit anti–mouse Ig (Becton Dickinson & Co., Heidelberg, Germany) and were analyzed by flow cytometry (FacScan®, Becton Dickinson & Co.). Immunostaining of frozen tissue sections of human tonsils was performed as described (7). Briefly, dried tissue sections were fixed for 3 min with acetone, incubated with mAbs for 1 h at room temperature, washed, and treated with affinity-purified peroxidase-conjugated rabbit anti–mouse Ig (P260; Dako Corp.). Antibody reactivity was visualized with 3-amino-9-ethylcarbazole (Sigma Chemical Co.) (0.25 mg/ml in 0.1 M acetate buffer, pH 5.0) with 0.003% H_2O_2. Tissue sections were counterstained with hemalaun.

Results

mAbs against CD19 and against TAPA-1 Selectively Induce Binding of B Cells to the Interfollicular Stroma of Tonsil Sections. To determine the adhesion pattern of B cells on tonsil sections and to identify B cell antigens that induce this binding, an in vitro adhesion assay on frozen tissue sec-

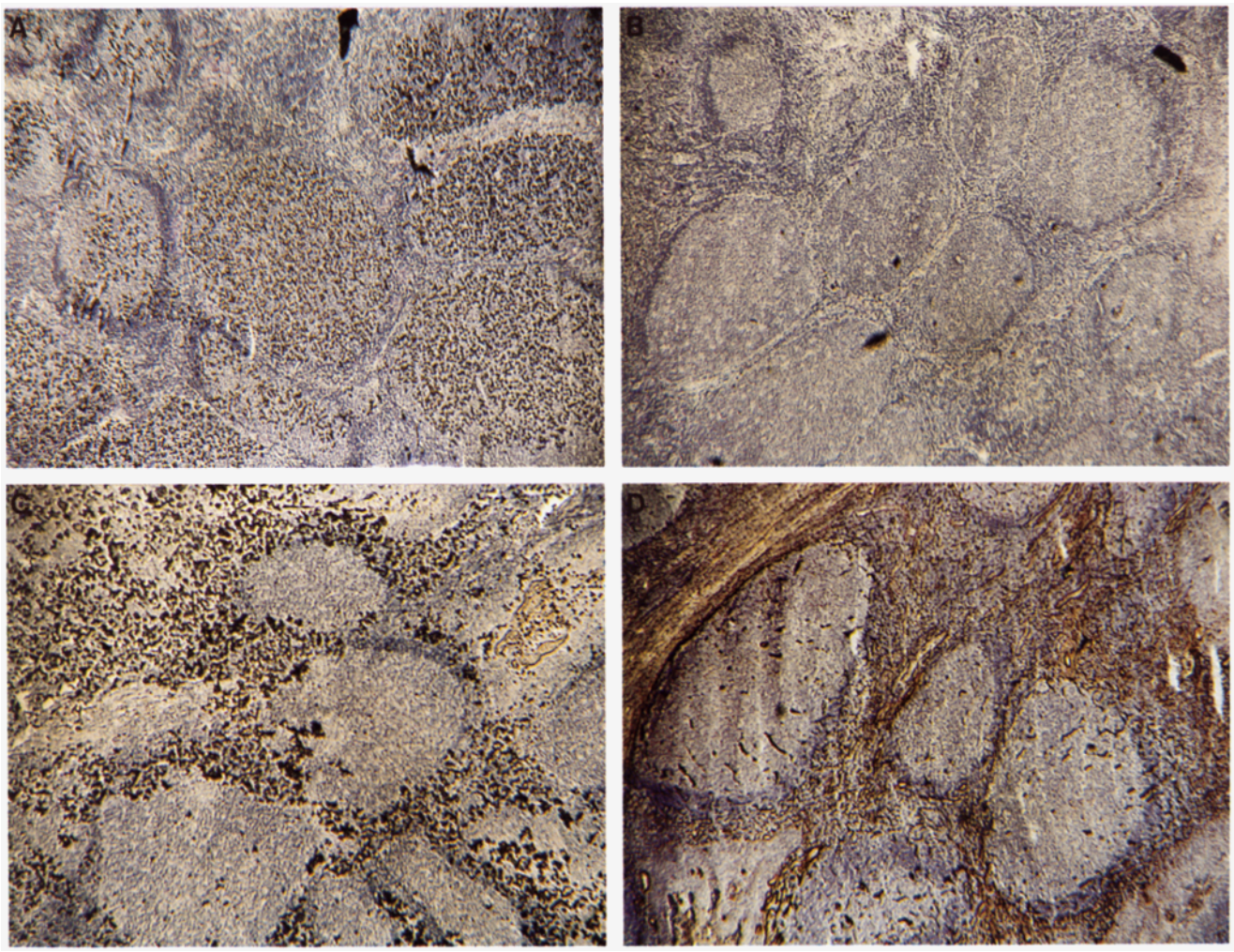

Figure 1. Treatment of B lymphocytes with anti-CD19 and anti–TAPA-1 mAbs induces binding of these cells to the FN-rich interfollicular stroma of tonsil sections. Adhesion assays were performed as described in Materials and Methods. ×25. (*A*) Untreated Reh cells bind to the germinal centers of lymphoid follicles. (*B*) Untreated Raji cells do not adhere to tonsil sections. (*C*) After incubation of Raji cells with mAbs against CD19, cells bind to the interfollicular stroma. The identical binding pattern of Raji cells was observed after treatment of cells with anti–TAPA-1 mAbs. (*D*) FN is expressed by the interfollicular stroma of tonsils as shown by immunoperoxidase staining with an anti-FN mAb.

tions was used. B cells were incubated under rotation on dried tonsil sections, which allowed these cells to adhere to tissue structures that could be identified under light microscopy. Whereas Reh and Nalm-6 cells bound to the germinal centers of lymphoid follicles (Fig. 1 *A* and Table 1), Raji, Ramos, Daudi, and normal human B cells did not bind to any area of the tonsils (Fig. 1 *B* and Table 1). To determine cell-surface molecules on B cells that were able to induce binding of B cells to tonsil sections, adhesion was tested after engaging cellular antigens with mAbs. Since untreated Raji cells did not adhere to tonsil sections, these cells were chosen to examine which mAbs promoted binding in situ. To test a broad range of mAbs that showed reactivity with Raji cells, a large number of available mAbs against a variety of cell-surface antigens with differing cell lineage specificity and function was screened for staining Raji cells by flow cytometry (data not shown). Among these Raji-reactive mAbs, only mAbs against CD19 and against TAPA-1 induced binding of Raji cells to tonsil sections, whereas the other mAbs tested did not initiate adherence (Table 2). The effect of anti-CD19 and anti–TAPA-1 mAbs was reduced in a graded manner with lower concentrations of these mAbs (data not shown). Induction of tissue binding, therefore, seemed to be a biologic reaction. An interesting observation was that mAbs against CD21, which, together with CD19, TAPA-1, and Leu-13, form a membrane complex on B cells (22–24, 30–33), did not induce adhesion in situ. Engaging IgM on Raji cells also had no effect on binding of these cells.

Cells treated with anti-CD19 showed the same binding pattern as cells labeled with anti–TAPA-1 mAbs: B cells incubated with either of the two mAbs bound exclusively to the interfollicular stroma of the tonsil but not to the germinal centers or to other parts of the lymphoid follicles (Fig. 1 *C*). The induction of adhesion was rapid, since incubation of cells with anti-CD19 or with anti–TAPA-1 mAbs for 5 min was sufficient to initiate binding (data not shown).

Next, we examined whether other B cell lines and nor-

Table 1. *mAb-dependent Binding Pattern of B Cells to Tonsil Sections and Antigen Expression of B Cells*

	B cells					
	Raji	Daudi	Reh*	Nalm-6*	Ramos	Normal B cells
Binding to tonsils after treatment						
Isotype control antibodies	−	−	−	−	−	−
anti-CD20	−	−	−	−	−	−
anti-CD19	IS	IS	IS	IS	−	−
anti-CD21	−	−	−	−	−	−
anti–TAPA-1 (CD81)	IS	IS	IS	IS	−	−
Cell surface antigen expression of untreated cells						
Isotype control antibodies	−	−	−	−	−	−
CD11a (LFA-1α)	+++	+++	++	++	++	++
CD19	++	+++	++	+++	+++	+++
CD20	+++	++	++	−	+++	++
CD21	+++	+++	+++	−	+	+++
CD29 (β1)	+++	+++	+++	+++	+	+
CD49c (α3)	−	−	−	−	+	+
CD49d (α4)	+++	+++	+++	+++	+	+
CD49e (α5)	−	−	−	+++	+	−
TAPA-1 (CD81)	++	+++	+	+++	+++	++
Act-1 (α4β7)	+	+++	++	+++	+	+

Cell surface antigen expression of untreated cells was determined by flow cytometry. The level of antigen expression was graded by the mean peak channel (−, ≤20; +, 21–115; ++, 116–200; +++, ≥201) on a three-log scale of fluorescence intensity.
*Reh and Nalm-6 cells also showed binding to the germinal centers of lymphoid follicles.
IS, binding to interfollicular stroma.

Table 2. *mAB-dependent Binding of Raji Cells to Human Tonsil Sections*

Binding to the interfollicular stroma	No binding
mAbs against CD19, TAPA-1 (CD81)	mAbs against IgM, CD10, CD11a (LFA-1α), CD18 (LFA-β2), CD20, CD21, CD22, CD23, CD29 (β1), CD32 (FcγRII), CD37, CD40, CD44, CD45RA, CD46, CD49d (α4), CD53, CD54 (ICAM-1), CD58 (LFA-3), CD70, CD72, CD102 (ICAM-2), Act-1 (α4β7)

An identical adhesion pattern was induced by five different anti-CD19 antibodies (J4-119, B43, BU12, HD237, HD37) and by two different TAPA-1 mAbs (5A6 and JS69). Since Raji cells expressed only IgM but not IgG, IgD, or IgA, induction of adhesion by anti-Ig was tested using an anti-IgM mAb. No adherence was exerted by isotype-matched control mAbs. Results shown are representative of at least three independent experiments.

mal B cells bound as well to tonsils after engaging CD19 or TAPA-1 with mAbs. Daudi, Reh, and Nalm-6 cells could also be induced to adhere to the interfollicular stroma by anti-CD19 or by anti–TAPA-1 mAbs but not by anti-CD20 or by anti-CD21 antibodies. The characteristic property of Reh and Nalm-6 cells to bind to the germinal center remained unaffected by antibody treatment, however (Table 1). Ramos cells and normal B cells, in contrast, did not show any binding to the tonsil sections after treatment with anti-CD19 or with anti–TAPA-1 mAbs (Table 1).

To investigate whether this lack of response of Ramos cells and normal B cells to the anti-CD19 or anti–TAPA-1 treatment could be correlated with low levels of distinct antigens, B cells were examined for expression of a panel of cell-surface molecules (Table 1). Whereas Ramos cells and normal B cells did not differ markedly from the other cell lines in their expression of CD19 or TAPA-1, they exhibited reduced levels of integrin α4 chain (CD49d) and integrin β1 chain (CD29).

Anti-CD19–induced Binding of Raji Cells to the Interfollicullar Stroma Involves Integrin α4β1 on the B Cell Surface. To identify adhesion molecules on Raji cells that were involved in the anti-CD19–induced binding, mAbs were examined for their ability to block tissue adherence of anti-CD19–treated cells (Table 3). For this purpose, anti-

Table 3. *Inhibition of Anti-CD19 mAb-induced Adhesion of Raji Cells to Interfollicular Stroma of Tonsil Sections by mAbs*

Complete	Partial	No inhibition of binding
mAb against CD49d (α4)	mAb against CD29 (β1)	mAbs against IgM, CD10, CD11a (LFA-1α), CD18 (LFA-β2), CD19, CD20, CD21, CD22, CD23, CD32 (FcγRII), CD37, CD40, CD44, CD45RA, CD46, CD49e (α5), CD53, CD54 (ICAM-1), CD58 (LFA-3), CD70, CD72, TAPA-1 (CD81), CD102 (ICAM-2), CD106 (VCAM-1), Act-1 (α4β7)

Inhibition of tissue binding of anti-CD19–treated cells by mAbs was determined relative to binding of anti-CD19–treated cells incubated with isotype-matched and B cell–reactive mAbs. Results shown are representative of at least three independent experiments.

CD19–labeled Raji cells were incubated with a panel of different B cell–reactive mAbs and were applied to tissue sections. Only antibodies against the integrin α4 chain and against the integrin β1 chain inhibited binding completely and partially, respectively, whereas the other mAbs tested did not impair adhesion in situ.

Anti-CD19 and anti–TAPA-1 mAbs Induce an Integrin α4–mediated Binding of B Cells to Purified Fibronectin. Next, we aimed at defining the receptor to which anti-CD19 and anti-TAPA-1–induced B cells were binding. The foregoing observations demonstrated that α4β1 on B cells was involved in the anti-CD19–induced binding to the interfollicular stroma. VCAM-1 (CD106) and the extracellular matrix (ECM) protein FN have been identified as ligands for α4β1 (34, 35). These antigens differ markedly in their tissue distribution, as shown by immunostaining. Consistent with earlier reports (20, 36), VCAM-1 was preferentially found on FDCs within the germinal centers (data not shown). FN, in contrast, was strongly expressed by stromal cells located in the interfollicular region of lymphoid follicles (Fig. 1 *D*). This tissue distribution of FN correlated closely with the areas where anti-CD19– and anti-TAPA-1–treated Raji cells adhered (Fig. 1, *C* and *D*). This led to the assumption that binding of α4β1 to one of its ligands, FN, was responsible for the in situ binding of Raji cells initiated by anti-CD19 and by anti–TAPA-1 mAbs. To verify this, we examined binding of mAb-treated B cells to purified FN.

To study the effect of antibody treatment on adhesion of B cells to purified FN, Raji and Ramos cells were treated with mAbs against CD19, CD20, CD21, or TAPA-1 and were examined for adhesion to plastic-immobilized FN (Fig. 2). FN- and α4-specific binding was defined by the ability to block adhesion to FN with an anti-FN serum and an anti-α4 mAb, respectively. Using this assay, anti-CD19– or anti-TAPA-1–treated Raji cells demonstrated an FN-specific binding that could also be blocked significantly by anti-α4. In contrast, anti-CD20– or anti-CD21–labeled Raji cells lacked FN/α4-specific adhesion. Ramos cells, treated with any of the mAbs, failed to show FN/α4-specific binding.

Induction of B Cell Binding to Purified Fibronectin by Anti-CD19 and by Anti–TAPA-1 mAbs Requires Different Intracellular Pathways. To identify the intracellular signals involved in the anti-CD19 and anti-TAPA-1–induced binding of Raji cells to purified FN, inhibitors of defined intracellular pathways were used (Fig. 3). Binding of anti-CD19 or anti-TAPA-1–treated Raji cells to FN was significantly reduced by genestein and herbimycin (inhibitors of tyrosine kinases) or by staurosporine (an inhibitor of protein kinases). In contrast, cycloheximide and H7 (inhibitors of protein synthesis and of protein kinase C, respectively) did not impair binding of anti-CD19– or anti-TAPA-1–labeled cells. A marked difference in inhibition of binding was observed when Raji cells, treated with anti-CD19 or with anti–TAPA-1 mAbs, were incubated with cytochalasin B (an inhibitor of the cytoskeleton). While binding of anti-CD19–treated cells to FN was strongly reduced by cytochalasin B, no effect was seen with this inhibitor on anti-TAPA-1–labeled Raji cells.

Discussion

The present study showed that engaging CD19 or TAPA-1 on human B lymphocytes with mAbs induced adhesion of these cells to the interfollicular stroma of human tonsils by initiating binding of integrin α4β1 to its ligand FN. Among a large number of B cell–reactive antibodies, only

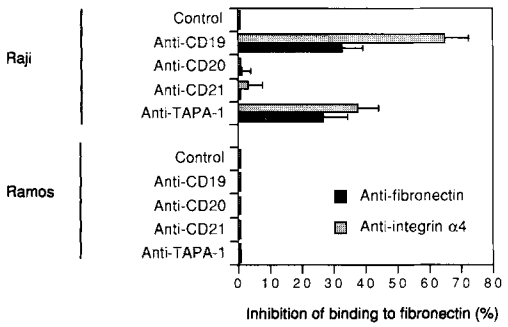

Figure 2. Raji cells treated with anti-CD19 and with anti–TAPA-1 mAbs adhere via α4 to immobilized FN. Specificity of binding was demonstrated using an anti-FN antiserum and an anti-α4 mAb to block binding sites on the FN layer and on the B cells, respectively. The percentage of inhibition by anti-FN and anti-α4 was determined relative to the number of adhering cells treated with negative control antibodies (antilaminin antiserum and anti-CD20 mAb, respectively). Results are the mean ± SD of three independent experiments.

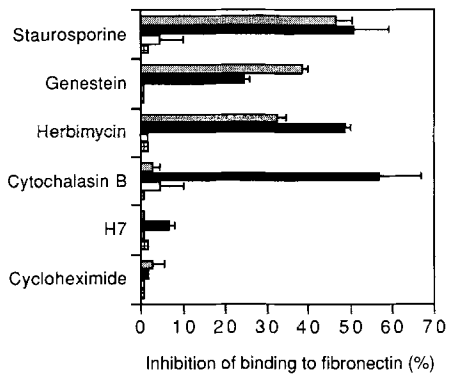

Figure 3. Requirements of intracellular pathways for anti-CD19– and anti–TAPA-1–induced adhesion of Raji cells to FN. Binding after engaging CD19 and TAPA-1 was reduced by inhibitors of tyrosine kinases (genestein and herbimycin) and by blockade of protein kinases (staurosporine), but not by an inhibitor of protein kinase C (H7) and not by inhibition of protein synthesis (cycloheximide). In contrast to anti-TAPA-1–treated cells, anti-CD19–incubated cells did not bind in the presence of an inhibitor of the cytoskeleton (cytochalasin B). The percentage of inhibition of binding was determined relative to the number of adhering cells in DMSO control medium (without inhibitors). Results are the mean ± SD of three independent experiments. ▦, control; □, anti-CD21; ■, anti-CD19; ▩, anti–TAPA-1 (CD81).

mAbs against CD19 or against TAPA-1 induced this binding phenomenon on Raji cells. Other B cell lines, i.e., Daudi, Nalm-6, and Reh cells, showed the same binding pattern, whereas Ramos cells and normal B cells failed to respond to incubation with anti-CD19 or anti–TAPA-1 mAbs. Using inhibitors of intracellular pathways, we could show that signals generated through either antigen, CD19 or TAPA-1, needed the action of tyrosine kinases to facilitate the α4β1/FN-mediated binding. Adhesion initiated by signaling through CD19 required an intact cytoskeleton, whereas binding induced by engaging TAPA-1 did not. This study demonstrates that the B cell surface molecules CD19 and TAPA-1 appear to have the unique and novel function of inducing an α4β1/FN-mediated adhesion of B cells to the interfollicular stroma of lymphoid tissues.

In the current study, a frozen section adhesion assay was used to identify B cell antigens that initiate binding of B cells to human tonsils. An important advantage of this assay was that it allowed detection of cellular binding to a tissue matrix that may resemble the microenvironment encountered by these cells in vivo. In fact, results obtained with this assay could be confirmed with experiments using physiologic conditions (29). In previous studies, this device was helpful to explain the adherence of leukocytes to high endothelial venules (28, 29) and to describe the adhesion of B cells to the germinal centers (20, 37, 38). Adhesion of B cells to germinal centers of lymphoid follicles, for example, was found to be mediated by α4β1 on the B cell surface and by VCAM-1 expressed on FDCs (20, 37). Since not all cell lines that were positive for α4β1 bound to the germinal center, the existence of other cellular structures that mediate this adhesion was postulated. The results obtained in the present study supported these earlier findings by showing that the cell lines Nalm-6 and Reh adhered to the germinal centers, while resting normal B cells and the B cell lines Raji, Ramos, and Daudi did not bind to these structures. These results and pilot studies using mAbs to block germinal center binding (38) were helpful, demonstrating that the frozen tissue assay used in our study was a valid method to detect adhesion through the α4β1 receptor.

CD19 and TAPA-1 have a central role for B cell physiology. These molecules, together with the complement receptor CD21 (CR2) and Leu-13, constitute a membrane complex on B cells that regulates several important B cell functions (22–24, 30–33). Each component of this complex influences independently the activation, proliferation, and function of B cells (22, 39). For example, cross-linking CD19 to membrane IgM on B cells lowered the threshold for antigen receptor–dependent stimulation of B cells (32). Other reports demonstrated that ligation of CD19 on B cells induced or blocked proliferation and antibody secretion, depending on the stimulating signal used (30). The CD21/CD19/TAPA-1 complex is relevant also for the adhesion of B cells, since engaging these molecules with mAbs induced homotypic binding. Whereas the anti-CD19–initiated aggregation occurred through LFA-1–dependent and –independent mechanisms (40, 41), the anti-TAPA-1–mediated binding was entirely independent of leukocyte function–associated antigen (LFA) 1 (24). However, homotypic adhesion could also be observed by engaging various other B cell antigens (41). Induction of aggregation, therefore, did not appear to be a phenomenon that was specific for signaling through the CD21/CD19/TAPA-1 complex.

Our observations provide evidence for a novel and unique role of CD19 and TAPA-1 in mediating B cell adhesion to ECM components. Among a large panel of mAbs tested, only mAbs against CD19 or against TAPA-1 induced binding of B cells to the interfollicular stroma of tonsils. This indicated that the CD21/CD19/TAPA-1 complex could have the specific function of initiating B cell adhesion to lymphoid stroma in situ. It was interesting to note that signaling through CD21 did not induce binding of B cells to tissue or to purified FN. This finding contrasts with observations that mAb binding to each component of the complex generated similar biologic effects by inducing cellular aggregation with identical morphology (23). Obviously, the α4β1/FN-mediated adhesion that was studied in our assays was different from homotypic adhesion and did not occur through signals generated via CD21. Furthermore, anti-CD19– and anti-TAPA-1–induced binding could also be observed with Nalm-6 cells that did not express CD21 (Table 1). It can be concluded, therefore, that the CD21 subunit of the CD21/CD19/TAPA-1 complex was not required to transmit signals that led to B cell binding via α4β1/FN.

To further dissect the signals generated by CD19 and TAPA-1, we defined the intracellular pathways that were necessary to mediate the α4β1/FN adhesion. Binding initiated through either molecule, CD19 or TAPA-1, involved the action of tyrosine kinases, since genestein and herbimycin inhibited adhesion after engaging CD19 or TAPA-1

with mAbs. Protein kinase C and protein synthesis were not involved in this signal transduction, since H7 and cycloheximide, respectively, did not affect cellular adhesion initiated by mAb binding to CD19 and TAPA-1. This signal transduction was similar, though, to the intracellular pathways that were involved in the homotypic adhesion initiated through several B cell antigens (41). We did not investigate, however, whether induction of α4β1/FN-mediated, heterotypic adhesion and initiation of homotypic adhesion are two separate events triggered by the same signal, i.e., engaging CD19 and TAPA-1 on the cell surface of B cells.

The current study identified integrin α4β1 as the receptor for the anti-CD19– and anti-TAPA-1–induced binding. Adhesion of anti-CD19–treated cells to tonsil sections could be inhibited completely by mAbs against the α4 chain and partially by mAbs against the β1 chain. Since the anti-α4 mAb abrogated binding more effectively than the anti-β1 mAb, it is possible that adhesion occurred mainly through the α4 chain of the α4β1 heterodimer complex. α4β7 and α4β1 contain the same α4 chain, and both bind to FN in vitro (42). An anti-α4β7 mAb did not reduce anti-CD19– and anti-TAPA-1–induced adhesion, however. It is possible that this antibody is directed against an epitope not involved in the anti-CD19– and anti-TAPA-1–induced binding to FN. Support for this interpretation comes from observations that distinct epitopes on α4β7 are responsible for adhesion of α4β7 to its different ligands, VCAM-1, mucosal addressin-1, and FN (43).

We could also demonstrate that FN was the ligand for binding of anti-CD19– and anti-TAPA-1–treated B cells. In contrast to Raji cells that were incubated with anti-CD20 or anti-CD21 mAbs or with isotype-control antibodies, only anti-CD19 and anti-TAPA-1–labeled cells showed FN-specific adhesion. In addition, this adhesion could be blocked using an anti-α4 mAb. Results of these binding experiments using purified FN were therefore consistent with the observations obtained in the frozen section assay that demonstrated an α4β1-specific tissue adherence only after engaging CD19 or TAPA-1 on the B cell surface. It was surprising to note that although Raji cells expressed α4β1 before antibody treatment, untreated cells did not adhere to the FN-rich interfollicular stroma of tonsils and also did not bind to purified FN. A quantitative change of the α4β1 receptor thus appears to be an unlikely cause for the anti-CD19– and anti-TAPA-1–induced binding. It seems plausible, instead, that on untreated B cells the adhesiveness of the α4β1 receptor for its ligand FN was too low to facilitate binding. Engaging CD19 or TAPA-1 with mAbs, in contrast, seems to significantly increase the avidity of α4β1/FN adhesion.

This hypothesis is supported by several reports about antigen-dependent modulation of β1-mediated adhesion to ECM components. Induction of binding of T cells to FN via β1 integrins could be observed, for example, by antigen stimulation (14), by signaling through CD50 (ICAM-3) (15), or by cross-linking the T cell receptor (17). In addition, adherence of leukocytes to ECM was induced by anti-β1 mAbs (44). Similarly, signaling via CD7 on NK cells induced a β1 integrin–mediated adhesion of these cells to FN (13). Since the expression levels of the β1 integrins remained unchanged after induction of binding, an increase of the avidity of the integrin receptor was considered to be the likely cause for the adhesion. Faull et al. (12) defined two distinct mechanisms that were responsible for an increase of β1-mediated binding of T cells to FN: First, affinity of the α4β1 receptor towards FN was increased by engaging β1 with an mAb. Second, treating the cells with PMA induced binding to FN by altering events that occurred after occupancy of the β1 receptor without affecting its affinity. These postreceptor events could be specifically blocked by cytochalasin D, an inhibitor of the cellular cytoskeleton. Likewise, modulation of the receptor affinity (45) and postreceptor events (46) have been described to be the basis for increased adhesion through the integrins αLβ2 (LFA-1, CD11a/CD18).

Data obtained in the present study support the interpretation that signaling through CD19 and TAPA-1 initiated two different models of increasing adhesiveness of α4β1/FN binding. Using cytochalasin B, an inhibitor of the cytoskeleton, we demonstrated that binding induced by engaging CD19 required the action of the cytoskeleton, whereas adhesion initiated via TAPA-1 was independent of the cytoskeleton. It is therefore likely that the signals generated through CD19 are involved in regulating postreceptor events. Engaging TAPA-1, in contrast, may lead to an increase of the affinity of α4β1 towards FN.

Several observations deserve further comment. The fact that Ramos cells and normal B cells did not respond to treatment with mAbs against CD19 and against TAPA-1 could not be explained by a lack of the two antigens on these cells. Instead, the expression levels for α4 and β1 were significantly lower on these cells than on the other B cells tested. It seems possible, therefore, that Ramos cells and normal B cells failed to demonstrate inducible binding via α4β1/FN because the amount of α4β1 on their cell surface did not permit adhesion through this receptor. Earlier findings that resting normal B cells also fail to adhere at the germinal center via α4β1/VCAM-1 (20) support the idea that appropriate stimuli or other yet undefined associated structures further influence the CD19 and TAPA-1–initiated binding through α4β1/FN.

It was also surprising to note that, although engaging CD19 or TAPA-1 induced α4β1-mediated binding of B cells to FN, germinal center binding via VCAM-1 remained unaltered on Reh and Nalm-6 cells and could not be induced on other cells (Table 1). A possible explanation for this observation might be that engaging CD19 and TAPA-1 initiated an adhesion of α4β1 to only one of its ligands, FN, whereas signaling through these and other B cell molecules had no effect on binding of α4β1 to its other ligand, VCAM-1.

Our observations provide grounds for a hypothetical model that may help to explain some new aspects of B cell physiology. These data represent the first description of a receptor-dependent regulation of B cell adhesion to the in-

terfollicular compartment of secondary lymphoid tissues. We propose that CD19 and TAPA-1 supply B cells with rapid and unique signals that result in temporary immobilization of B cells within the stromal matrix. By binding to ECM components, these cells may receive further signals that define their differentiation and function (11). Within this microenvironment, adhering B cells might also form contact with T cells of the interfollicular area and could thereby influence the function of these T cells. Recently, CD77 has been identified as a potential ligand for CD19 (47). Since CD77 is constitutively expressed on germinal center B cells entering apoptosis (48), the CD19–CD77 interaction may have an important impact on the fate of B cells in secondary lymphoid organs. Future studies will focus on how receptor-regulated adhesion of B cells to ECM determines B cell function.

We thank Drs. Shoshana Levy, Timothy Carlos, and Andrew Lazarovitz for the gift of anti–TAPA-1, anti–VCAM-1, and anti-α4β7 mAbs, respectively; Dr. Stefan Serke for help in performing flow cytometry; Drs. Markus Mapara, Bernd Dörken, Michael Hummel, and Christian Schmidt for supply of cell lines; Drs. Carolin Schriever and Heike Pahl for comments on the manuscript; and Dr. Irene Boll for the generous donation of a high resolution light microscope. F. Schriever dedicates this work to his scientific mentors, Drs. Judith Johnson, Gert Riethmüller, Lee Nadler, Marshall Wolf, and Dieter Huhn.

This work was supported by grant Schr 318/2-1 of the Deutsche Forschungsgemeinschaft, by the Berliner Krebsgesellschaft, and by the Maria Sonnenfeld Stiftung (Berlin).

Address correspondence to Dr. Folke Schriever, Department of Hematology and Oncology, Virchow University Hospital, Humboldt University Berlin, Augustenburger Platz 1, 13353 Berlin, Germany.

Received for publication 8 March 1995 and in revised form 23 May 1995.

References

1. Springer, T.A. 1994. Traffic signals for lymphocyte recirculation and leukocyte emigration: the multistep paradigm. *Cell.* 76:301–314.
2. Picker, L.J., and E.C. Butcher. 1992. Physiological and molecular mechanisms of lymphocyte homing. *Annu. Rev. Immunol.* 10:561–591.
3. Steinman, R.M., and Z.A. Cohn. 1973. Identification of a novel cell type in peripheral lymphoid organs of mice. I. Morphology, quantitation, tissue distribution. *J. Exp. Med.* 137:1142–1151.
4. Steinman, R.M., B. Gutchinov, M.D. Witmer, and M.C. Nussenzweig. 1983. Dendritic cells are the principal stimulators of the primary mixed leukocyte reaction in mice. *J. Exp. Med.* 157:613–627.
5. Clark, E.A., and J.A. Ledbetter. 1994. How B and T cells talk to each other. *Nature (Lond.).* 367:425–428.
6. Liu, Y.-J., D.E. Joshua, G.T. Williams, C.A. Smith, J. Gordon, and I.C.M. MacLennan. 1989. Mechanism of antigen-driven selection in germinal centres. *Nature (Lond.).* 342:929–931.
7. Schriever, F., A.S. Freedman, G. Freeman, E. Messner, G. Lee, J. Daley, and L.M. Nadler. 1989. Isolated human follicular dendritic cells display a unique antigenic phenotype. *J. Exp. Med.* 169:2043–2058.
8. Schriever, F., and L.M. Nadler. 1992. The central role of follicular dendritic cells in lymphoid tissues. *Adv. Immunol.* 51: 243–284.
9. Schnizlein, C.T., M.H. Kosco, A.K. Szakal, and J.G. Tew. 1985. Follicular dendritic cells in suspension: identification, enrichment, and initial characterization indicating immune complex trapping and lack of adherence and phagocytic activity. *J. Immunol.* 134:1360–1368.
10. Liu, Y.-J., G.D. Johnson, J. Gordon, and I.C.M. MacLennan. 1992. Germinal centres in T-cell-dependent antibody responses. *Immunol. Today.* 13:17–21.
11. Shimizu, Y., and S. Shaw. 1991. Lymphocyte interactions with extracellular matrix. *FASEB J.* 5:2292–2299.
12. Faull, R.J., N.L. Kovach, J.M. Harlan, and M.H. Ginsberg. 1994. Stimulation of integrin-mediated adhesion of T lymphocytes and monocytes: two mechanisms with divergent biological consequences. *J. Exp. Med.* 179:1307–1316.
13. Rabinowich, H., W.-C. Lin, R.B. Herbermann, and T.L. Whiteside. 1994. Signalling via CD7 molecules on human NK cells: induction of tyrosine phosphorylation and β1 integrin-mediated adhesion to fibronectin. *J. Immunol.* 153:3504–3513.
14. Chan, B.M.C., J.G.P. Wong, A. Rao, and M.E. Hemler. 1991. T cell receptor-dependent, antigen-specific stimulation of a murine T cell clone induces a transient, VLA protein-mediated binding to extracellular matrix. *J. Immunol.* 147: 398–404.
15. Cid, M.C., J. Esparza, M. Juan, A. Miralles, J. Ordi, R. Vilella, A. Urbano-Marquez, A. Gaya, J. Vives, and J. Yague. 1994. Signaling through CD50 (ICAM-3) stimulates T lymphocyte binding to human umbilical vein endothelial cells and extracellular matrix proteins via an increase in β1 and β2 function. *Eur. J. Immunol.* 24:1377–1382.
16. Salmon, D.R., C.F. Mojcik, A.C. Chang, S. Wadsworth, D.H. Adams, J.E. Coligan, and E.M. Shevach. 1994. Constitutive activation of integrin α4β1 defines a unique stage of human thymocyte development. *J. Exp. Med.* 179:1573–1584.
17. Gijs, Y., G.A. Van Seventer, K.J. Horgan, and S. Shaw. 1990. Regulated expression and binding of three VLA (β1) integrin receptors on T cells. *Nature (Lond.).* 345:250–253.
18. Dustin, M.L., and T.A. Springer. 1989. T-cell receptor cross-linking transiently stimulates adhesiveness through LFA-1. *Nature (Lond.).* 341:619–624.
19. Van Kooyk, Y., P. van de Wiel-van Kememade, P. Weder, T.W. Kuijpers, and C.G. Figdor. 1989. Enhancement of LFA-1-mediated cell adhesion by triggering through CD2 or CD3 on T lymphocytes. *Nature (Lond.).* 342:811–813.

20. Freedman, A.S., J.M. Munro, G.E. Rice, P. Bevilacqua, C. Morimoto, B.W. McIntyre, K. Rhynhart, J.S. Pober, and L.M. Nadler. 1990. Adhesion of human B cells to germinal centers in vitro involves VLA-4 and INCAM-110. *Science (Wash. DC).* 249:1030–1033.
21. Chen, Y.X., K. Welte, D.H. Gebhard, and R.L. Evans. 1984. Induction of T cell aggregation by antibody to a 16kd human leukocyte surface antigen. *J. Immunol.* 133:2496–2501.
22. Matsumoto, A.K., D.R. Martin, R.H. Carter, L.B. Klickstein, J.M. Ahern, and D.T. Fearon. 1993. Functional dissection of the CD21/CD19/TAPA-1/Leu-13 complex of B lymphocytes. *J. Exp. Med.* 178:1407–1417.
23. Bradbury, L.E., G.S. Kansas, S. Levy, R.T. Evans, and T.F. Tedder. 1992. The CD19/CD21 signal transducing complex of human B lymphocytes includes the target of antiproliferative antibody-1 and Leu-13 molecules. *J. Immunol.* 149: 2841–2850.
24. Takahashi, S., C. Doss, S. Levy, and R. Levy. 1990. TAPA-1, the target of an antiproliferative antibody, is associated on the cell surface with the Leu-13 antigen. *J. Immunol.* 145:2207–2213.
25. Oren, R., S. Takahashi, C. Doss, R. Levy, and S. Levy. 1990. TAPA-1, the target of an antiproliferative antibody, defines a new family of transmembrane proteins. *Mol. Cell. Biol.* 10: 4007–4015.
26. Lazarovits, A.I., and J. Karsh. 1993. Differential expression in rheumatoid synovium and synovial fluid of $\alpha4\beta7$ integrin. *J. Immunol.* 11:6482–6489.
27. Carlos, T.M., B.R. Schwartz, N.L. Kovach, E. Yee, M. Rosso, L. Osborn, G. Chi-Rosso, B. Newman, R. Lobb, and J.M. Harlan. 1990. Vascular cell adhesion molecule-1 mediates lymphocyte adherence to cytokine-activated cultured human endothelial cells. *Blood.* 76:965–970.
28. Stamper, H.B.J., and J.J. Woodruff. 1976. Lymphocyte homing into lymph nodes: in vitro demonstration of the selective affinity of recirculating lymphocytes for high endothelial venules. *J. Exp. Med.* 144:828–833.
29. Butcher, E.C., R.G. Scollay, and I.L. Weissman. 1979. Lymphocyte adherence to high endothelial venules: characterization of a modified in vitro assay, and examination of the binding of syngeneic and allogeneic lymphocyte populations. *J. Immunol.* 123:1996–2003.
30. Callard, R.E., K.P. Rigley, S.H. Smith, S. Thurstan, and J.G. Shields. 1992. CD19 regulation of human B cell responses. B cell proliferation and antibody secretion are inhibited or enhanced by ligation of the CD19 surface glycoprotein depending on the stimulating signal used. *J. Immunol.* 148:2983–2987.
31. Hebell, T., J.M. Ahearn, and D.T. Fearon. 1991. Suppression of the immune response by a soluble complement receptor of B lymphocytes. *Science (Wash. DC).* 254:102–105.
32. Carter, R.H., and D.T. Fearon. 1992. CD19: lowering the threshold for antigen receptor stimulation of B lymphocytes. *Science (Wash. DC).* 256:105–107.
33. Pezzuitto, A., B. Dörken, P.S. Rabinovitch, J.A. Ledbetter, G. Moldenhauer, and E.A. Clark. 1987. CD19 monoclonal antibody HD37 inhibits anti-immunoglobulin-induced B cell activation and proliferation. *J. Immunol.* 138:2793–2799.
34. Pulido, R., M.J. Elices, M.R. Campanero, L. Osborn, S Schiffer, A. Garcia-Pardo, R. Lobb, M.E. Hemler, and F. Sanchez-Madrid. 1991. Functional evidence for three distinct and independently inhibitable adhesion activities mediated by the human integrin VLA-4. *J. Biol. Chem.* 266:10241–10248.
35. Elices, M.J., L. Osborn, Y. Takada, C. Crouse, S. Luhowskyj, M.E. Hemler, and R.R. Lobb. 1990. VCAM-1 on activated endothelium interacts with the leukocyte integrin VLA-4 at a site distinct from the VLA-4/fibronectin binding site. *Cell.* 60:577–584.
36. Rice, G.E., J.M. Munro, and M.P. Bevilacqua. 1990. Inducible cell adhesion molecule 110 (INCAM-110) is an endothelial receptor for lymphocytes. A CD11/CD18-independent adhesion molecule. *J. Exp. Med.* 171:1369–1374.
37. Freedman, A.S., J.M. Munro, C. Morimoto, B.W. McIntyre, K. Rhynhart, N. Lee, and L.M. Nadler. 1992. Follicular non-Hodgkin's lymphoma cell adhesion to normal germinal centers and neoplastic follicles involves very late antigen-4 and vascular cell adhesion molecule-1. *Blood.* 79:206–212.
38. Behr, S.I., D. Huhn, and F. Schriever. 1995. Adhesion molecules involved in the binding of B cell lines to the human germinal center. *In* Leucocyte Typing V. Oxford University Press, Oxford, UK. 1632–1634.
39. Matsumoto, A.K., J. Kopicky-Burd, R.H. Carter, D.A. Tuveson, T.F. Tedder, and D.T. Fearon. 1991. Intersection of the complement and immune systems: a signal transduction complex of the B lymphocyte containing complement receptor type 2 and CD19. *J. Exp. Med.* 173:55–64.
40. Smith, S.H., K.P. Rigley, and R.E. Callard. 1991. Activation of human B cells through the CD19 surface antigen results in homotypic adhesion by LFA-1-dependent and -independent mechanisms. *Immunology.* 73:293–297.
41. Kansas, G.S., and T.F. Tedder. 1991. Transmembrane signals generated through MHC class II, CD19, CD20, CD30, and CD40 antigens induce LFA-1-dependent and independent adhesion in human B cells through a tyrosine kinase-dependent pathway. *J. Immunol.* 147:4092–4102.
42. Ruegg, C., A.A. Postigo, E.E. Sikorski, E.C. Butcher, R. Pytela, and D. Erle. 1992. Role of integrin $\alpha4/\beta7/\alpha4/\beta P$ in lymphocyte adherence to fibronectin and VCAM-1 and in homotypic cell clustering. *J. Cell Biol.* 117:179–189.
43. Andrew, D.P., C. Berlin, S. Honda, T. Yoshino, A. Hamann, B. Holzmann, P.I. Kishaw, and E.C. Butcher. 1994. Distinct but overlapping epitopes are involved in $\alpha4\beta7$-mediated adhesion to vascular cell adhesion molecule-1, mucosal addressin-1, fibronectin, and lymphocyte aggregation. *J. Immunol.* 153:3847–3861.
44. Kovach, N.L., T.M. Carlos, E. Yee, and J.M. Harlan. 1992. A monoclonal antibody to $\beta1$ integrin (CD29) stimulates VLA-dependent adherence of leukocytes to human umbilical vein endothelial cells and matrix components. *J. Cell Biol.* 116:499–509.
45. Hibbs, M.L., H. Xu, S.A. Stacker, and T.A. Springer. 1991. Regulation of adhesion to ICAM-1 by the cytoplasmic domain of LFA-1 integrin β subunit. *Science (Wash. DC).* 251: 1611–1613.
46. Peter, K., and T.E. O'Toole. 1995. Modulation of cell adhesion by changes in $\alpha_L\beta_2$ (LFA-1, CD11a/CD18) cytoplasmic domain/cytoskeleton interaction. *J. Exp. Med.* 181:315–326.
47. Maloney, M.D., and C.A. Lingwood. 1994. CD19 has a potential CD77 (globotriaosyl ceramide)-binding site with sequence similarity to verotoxin B-subunits: implications of molecular mimicry for B cell antigen and enterohemorrhagic *Escherichia coli* pathogenesis. *J. Exp. Med.* 180:191–201.
48. Mangeney, M., Y. Richard, D. Coulaud, T. Tursz, and J. Wiels. 1991. CD77: an antigen of germinal center B cells entering apoptosis. *Eur. J. Immunol.* 21:1131–1140.