Signaling and Induction of Enhanced Cytoadhesiveness Via the Hematopoietic Progenitor Cell Surface Molecule CD34

By Otto Majdic, Johannes Stöckl, Winfried F. Pickl, Jan Bohuslav, Herbert Strobl, Clemens Scheinecker, Hannes Stockinger, and Walter Knapp

The transmembrane glycoprotein CD34 shows a highly restricted expression on a crucial subset of hematopoietic cells. We show here that engagement of particular determinants of CD34 can lead to signal transduction and to enhanced adhesiveness of CD34+ hematopoietic cells. Monoclonal antibodies (MoAbs) directed against O-sialylglycoprotease-sensitive epitopes of CD34 (OBEND10, ICH3, BI.3C5, MY10) but not MoAbs against O-sialylglycoprotease-resistant epitopes (9F2, BG12) induce actin polymerization in KG-1a and KG-1 cells and strongly enhanced cytoadhesiveness. The capacity to induce adhesion requires cellular energy, divalent cations, and intact cytoskeleton but not de novo protein synthesis. The observed cytoadhesion seems at least in part to be caused by a concomitant activation of the β2 integrin cytoadhesion pathway. It can be significantly inhibited with lymphocyte function-associated antigen-1 and intercellular adhesion molecule-1 antibodies. Protein kinase inhibition analyses suggest that the pathways initiated by engagement of the CD34 molecule with certain CD34 MoAbs involves protein tyrosine kinases but that protein kinase C is not critically involved.

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land). The dye 2',7'-bis(2-carboxyethyl)-5-(and-6)-carboxyfluorescein, acetoxymethyl ester (BCECF-AM) for vital cell staining and N-(7-nitrobenz-2-oxa-1,3-diazol-4-yl)-phallacidin (NBD-Phallacidin) for F-actin detection were obtained from Molecular Probes, Inc (Eugene, OR). Neuraminidase from Vibrio cholerae (VCN) was obtained from Behring AG (Marburg, Germany). O-sialoglycoprotease from Fusobacteria haemolytica was provided by Dr A. Mellors, Ontario, Canada.

Act D and cycloheximide concentrations used were tested in parallel experiments by monitoring 3H-Uridine or 3H-Leucine uptake by KG-1a cells. Marked and specific inhibition of 3H-incorporation showed effectiveness of these substances for inhibition of transcription or protein synthesis, respectively, in our system.

MoAbs. The following murine CD34 MoAbs were used in this study: MoAb QBEND10 (IgG1; Quantum, Biomed, London, UK), ICH-3 (IgG2a; Applied Immune Sciences, Inc, Menlo Park, CA), Bl.1.C5 (IgG2b; IVth Workshop and Conference on Human Leucocyte Differentiation Antigens, Vienna, Austria), MY10 (HPCA1, IgG2b) and 8G12 (HPCA2, IgG1) (Becton Dickenson, Mountain View, CA) and 9F2 (established in our institute). MoAb 9F2 (isotype IgG1) was obtained after immunization with KG-1a cells using immunization, fusion, and cloning protocols essentially as described previously.

The molecular specificity was identified in immunoprecipitation experiments and with CD34-immunoglobulin fusion proteins (established in our institute according to Aruffo et al11). For cytoadhesion inhibition studies, we used the CD11a MoAbs SE6 and 6B7 (our laboratory); the CD11b MoAb LP19c (supplied by Dr K. Pulford, Oxford, UK); the CD11c MoAb S-HCL-3 (supplied by Dr R. Schawarting, Philadelphia, PA); the CD18 MoAbs M232 (obtained from the IVth Workshop and Conference on Human Leucocyte Differentiation Antigens, MHM23 (supplied by Dr A.J. McMichael, Oxford, UK), the CD54 MoAb RR1/1, which was obtained from Dr R. Rothlein (Ridgefield, CT), the MoAbs HP2.1 (CDw49d), SAM1 (CDw49e) obtained from Immunootech (Marseille, France), MoAb 4B4 (CD29, Couter Immunology, Hialeah, FL) the MoAb TS2/9 (CD58, hybridoma obtained from the American Tissue Culture Collection, ATCC, Rockville, MD) and MoAb 1B5 (CD31, our institute). As controls, we used MoAb 6B6 (CD44), MoAbs VIP1 (CD71), AAa6 (M6 antigen18), and 3B2 (EZ-antigen19), all established in our laboratory, and all are strongly reactive with KG-1a and KG-1a cells. The nonbinding isotype control MoAb VIAP was established in our laboratory.

Cells. The human cell lines used in this study, KG-1, KG-1a, HL-60, and Daudi were obtained from ATCC and were cultured in simplified medium (SM, 145 mmol/L NaCl, 0.75 mmol/L KCl, 15 mmol/L glucose, 10 mmol/L HEPES, pH 7.40) and resuspended in 5% fetal calf serum (FCS), humidified atmosphere at 37°C. Cell viability and potential toxicity on treatment of KG-1a cells and Daudi Cells in the amount of 5 x 106 were incubated for 30 minutes at 37°C with VCN (10 mM/L) or purified O-sialoglycoprotease from P haemolytica (0.06 mg/mL),20 and then assayed by immunofluorescence staining and flow cytometry.

Adhesion assays. A modification of the semiquantitative aggregation method described by Rothlein and Springer22 for the analysis of homotypic aggregate formation was used. Fifty microliters of MoAb (20 mg/mL) or RPMI 1640 medium and 100 mL of cell suspension (1.25 x 105/mL) were added to flat-bottomed 96-well microtiter plates (Cel-Cult, Sterilin Ltd, Hounsow, UK). After moderate shaking, cells were allowed to settle and the degree of aggregation was scored after 90 minutes or otherwise at indicated time points. Scores ranged from 0 to +4. + 0 indicated 10% of the cells were in aggregates; +1 represented 10% to 50% of the cells in aggregated form; +2, about 50% to 75% of the cells were aggregated; +3, up to 100% of the cells were aggregated; and +4 indicated that 100% of the cells were in large, tightly bound aggregates.

Plastic adherence of KG-1a cells to microtiter plates, which was performed to test for effectiveness of drug concentrations applied in our experiments, was monitored on pretreatment of cells with the indicated protein kinase inhibitors or medium alone and subsequent addition of 10-7 mol/L PMA. The nonadherent and loosely attached cells were removed by three consecutive washing steps with medium. Binding of cells was quantitated by counting the number of adherent cells per well in relation to the total number of cells added to each well.

Analysis of heterotypic cell adhesion (rosette formation assay). In coaggregation experiments of KG-1 cells (1 x 105/mL) with Daudi or HL60 cells (1 x 105/mL), KG-1 cells were stained with BCECF-AM at 37°C for 30 minutes, washed twice, and incubated at 4°C with MoAb QBEND10 (CD34). Subsequently, aliquots of both cell suspensions were mixed and centrifuged at 50g for 5 minutes. Afterwards, the cells were incubated for 2 hours at 37°C. Heterotypic cell adhesion of KG-1 cells and Daudi or HL-60 cells induced by MoAb QBEND10 was analyzed using an inverted fluorescence microscope (Leitz Aristoplan, Jena, Germany). By enumerating 100 fluorescent-labeled (+) KG-1 cells, heterogregarate events were regarded as positive when three or more nonfluorescent (-)–labeled adhered to one + KG-1 cell.

Measurement of intracellular free calcium. Determinations of free intracellular calcium levels were performed according to Grynkiewicz et al23 with slight modifications. KG-1a (1 x 105 cells/mL) cells were suspended in RPMI 1640 + 10% FCS and incubated with the Ca2+-sensitive probe Fura-2 (1 mmol/L) (Molecular Probes, Inc) for 30 minutes at 37°C. An aliquot of 3 x 106 cells was washed twice in simplified medium (SM, 145 mmol/L NaCl, 5 mmol/L KCl, 1 mmol/L Na2HPO4, 1 mmol/L CaCl2, 0.5 mmol/L MgSO4, 5 mmol/L glucose, 10 mmol/L HEPS, pH 7.40) and resuspended in 2 mL of SM and transferred to a quartz glass cuvette. MoAbs were added to give a final concentration of 30 mg/mL. As a positive control, the Ca2+ ionophore A23187 (0.75 mmol/L) was used. In cross-linking experiments, Fura-2--labeled cells were incubated with the indicated MoAb (10 mg/mL). After 30 minutes, aliquots of 3 x 106 cells were washed three times with SM and resuspended in 2 mL SM. SAM was added at 25 mg/mL to cross-link cell surface-bound MoAb. Fluorescence intensity (FI) was measured on a spectrofluorometer (Perkin Elmer, Norwalk, CT). Concentrations of free cytoplasmic calcium levels were calculated according to the formula described by Grynkiewicz et al.22
**Results**

**Induction of homotypic adhesion via the CD34 molecule.** Searching for potential functional effects that can be induced or mediated via the CD34 molecule, we incubated cells of the CD34+ hematopoietic cell line KG-1a with six different CD34 antibodies and examined their effects on the morphology and growth characteristics of KG-1a cells at different time points. We observed that the CD34 antibodies QBEND10 and ICH3 can induce very pronounced homoaggregate formation of KG-1a cells, whereas two other CD34 MoAbs (MY10, BL3C5) have less dramatic but clearly detectable adhesion-inducing effects and two CD34 MoAbs (8G12 and 9F2) were completely ineffective in this respect (Fig 1A). Typical examples of the observed aggregation patterns are shown in Fig 2.

The capacity to induce enhanced adhesion of KG-1a cells did not correlate with the fluorescence intensity of KG-1a cells after staining with these MoAbs (Fig 1B). Indeed, the strongest binding MoAb (8G12) was unable to trigger homotypic adhesion. In addition, binding of a panel of antibodies to other surface molecules strongly expressed by KG-1a cells including the CD44 MoAb 6B6, the CD71 MoAb VIP1, the anti-M6 MoAb AAA6,18 and the anti-E2 MoAb 3B2 did not induce adhesion. Titration experiments showed that a QBEND10 MoAb concentration of 0.6 μg/ml is sufficient to induce detectable homoaggregate formation (Fig 3A).

The adhesion-inducing capacity seems to correlate clearly with the region of the CD34 molecule to which individual CD34 antibodies bind (Table 1). The determinants recognized by the four adhesion inducing CD34 antibodies are all exquisitely sensitive to P haemolytica O-sialoglycoprotease treatment. MoAbs ICH3, BL3C5, and MY10 are also sensitive to a variable degree to treatment with neuraminidase from V cholerae. Quite in contrast, the two non-adhesion-inducing CD34 antibodies recognize determinants that are not at all sensitive to these two enzymes, but become even better accessible to antibody binding, a finding that is in accord with observations made by Sutherland et al classifying MoAb 8G12 as being specific for a class III epitope of the CD34 moiety on KG-1 cells.26 Cross-inhibition studies show that the two prototype antibodies QBEND10 (aggregation inducing) and 8G12 (not aggregation inducing) bind to clearly distinct epitopes and show a heterogeneous inhibition pattern for the other antibodies (Table 1).

Experiments performed with O-sialoglycoprotease–treated KG-1a cells confirmed that antibody binding to the O-sialoglycoprotease–sensitive portion of CD34 is essential. The
enhanced binding of the CD34 MoAbs 8G12 and 9F2 to the O-sialoglycoproteinase–resistant CD34 portion did not induce aggregation (data not shown).

Requirement for cellular energy, divalent cations, and an intact cytoskeleton. We next investigated the metabolic requirements of adhesion induction.

As shown in Table 2, adhesion induced by MoAb QBEND10 was also completely ablated by metabolic depletion of cellular adenosine triphosphate and concomitant inhibition of phosphatases\(^1\) by prior incubation with NaF\(^7\) or by chelation of cationic ions with EDTA. Paralysis of the cytoskeleton by cytochalasin B completely abrogated adhesion induced by MoAb QBEND10. However, adhesion was not affected by cycloheximide or Act D used in concentrations able to inhibit \(^1^H\)-leucine or \(^1^H\)-uridine uptake of KG-1a cells, respectively. This suggests that de novo protein synthesis was not required for homotypic adhesion. Adhesion induction by CD34 MoAb QBEND10 was found to be strictly dependent on temperature with aggregation occurring after 30 minutes and being maximal at 90 minutes (Fig 3B). At 0°C, no aggregate formation can be induced.

Thus, the observed binding and aggregation patterns argued against the possibility of a mere passive agglutination of KG-1a cells by certain CD34 MoAbs.

Induction of actin polymerization. Shape changes, membrane ruffling, and enhanced cytoadhesiveness that accompany cell activation are dependent on the assembly and reorganization of the actin cytoskeleton. As shown above,
Table 1. Epitope Mapping of CD34 MoAbs Under Study

<table>
<thead>
<tr>
<th>MoAbs</th>
<th>O-sialo Glycoprotease</th>
<th>V.cholerae Neuraminidase</th>
<th>QBEND10 Binding</th>
<th>BG12 Binding</th>
</tr>
</thead>
<tbody>
<tr>
<td>QBEND10</td>
<td>-100*</td>
<td>+13*</td>
<td>971</td>
<td>01</td>
</tr>
<tr>
<td>ICH3</td>
<td>-100</td>
<td>-67</td>
<td>64</td>
<td>39</td>
</tr>
<tr>
<td>Bl.3C5</td>
<td>-100</td>
<td>-98</td>
<td>65</td>
<td>9</td>
</tr>
<tr>
<td>MY10</td>
<td>-100</td>
<td>-60</td>
<td>19</td>
<td>1</td>
</tr>
<tr>
<td>9F2</td>
<td>+60</td>
<td>+65</td>
<td>40</td>
<td>33</td>
</tr>
<tr>
<td>BG12</td>
<td>+44</td>
<td>+29</td>
<td>1</td>
<td>97</td>
</tr>
</tbody>
</table>

* Percent change in MFI after enzyme treatment of KG-1a cells.
† Percent inhibition of FITC-conjugated QBEND10 or BG12 MoAbs binding to KG-1a cells after preincubation with indicated MoAb.

Table 2. CD34 MoAb-Induced Homotypic Adhesion Requires Cellular Energy, Divalent Cations, and an Intact Cytoskeleton

<table>
<thead>
<tr>
<th>Inhibitors</th>
<th>Adhesion Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>NaF</td>
<td>0+</td>
</tr>
<tr>
<td>EDTA</td>
<td>0+</td>
</tr>
<tr>
<td>CytoB</td>
<td>0+</td>
</tr>
<tr>
<td>Cyclohex</td>
<td>3+</td>
</tr>
<tr>
<td>Act D</td>
<td>3+</td>
</tr>
<tr>
<td>None</td>
<td>3+</td>
</tr>
</tbody>
</table>

KG-1a cells were preincubated with inhibitors for 30 minutes at 37°C before initiation of the adhesion assay by adding CD34 MoAb QBEND10 (final concentration 5 μg/mL). Identical results were obtained in three independent experiments. Inhibitors were used as follows: NaF 30 mmol/L, EDTA (5 mmol/L), cytochalasin B (CytoB; 10 μg/mL), cycloheximide (Cyclohex; 1 μg/mL), and actinomycin D (Act D; 1 μg/mL).

The invoked adhesiveness of KG-1a cells by MoAb QBEND10 binding was completely abolished when cytochalasin B, which inhibits microfilament formation, was added. To ascertain the involvement of reorganized cytoskeletal microfilaments in the observed adhesion of KG-1a cells after stimulation with certain CD34 MoAbs, we analyzed the F-actin content in stimulated and unstimulated cells. As can be seen from Fig 4A, CD34 MoAb QBEND10 indeed induces actin polymerization in KG-1a cells and significantly enhanced F-actin staining. The observed increases in F-actin content measured as RFI are not as dramatic as that observed after stimulation with PMA known to induce actin polymerization in various cell types, but are clearly detectable. No such effects were obtained with the CD34 MoAb 9F2 or CD44 MoAb 6B6.

**Effects of CD34 MoAbs on intracellular calcium levels.** Mobilization of free cytoplasmic calcium often accompanies receptor-mediated cellular activation. However, with the stimulatory CD34 MoAbs only minute changes in intracellular Ca²⁺ concentrations could be detected (data not shown). They were in the same range as that observed after incubation with a nonstimulatory CD34 MoAb 9F2 and could not be enhanced by cross-linkage with SAM. In both experimental settings, the cells responded to the Ca²⁺ ionophore A23187 but not to negative control MoAbs.

**Effect of protein kinase inhibitors on CD34 MoAb-induced cell aggregation.** To find out if the intracellular signaling pathway for CD34 mediated KG-1a cell aggregation induction involves activation of PKC or tyrosine kinase activity, we used the tyrosine kinase inhibitor herbimycin A and the PKC inhibitors staurosporine and H7 and analyzed the influence of these agents on CD34 MoAb-induced aggregate formation. Herbimycin A (5 μmol/L) significantly diminished the aggregation response of KG-1a cells on MoAb QBEND10 binding. Neither of the two PKC inhibitors (H7, staurosporine) influenced MoAb QBEND10–induced cell clustering (Table 3). None of the substances used at the indicated concentrations affected cell viability as determined by propidium iodide exclusion followed by FACS analyses.

Involvement of lymphocyte function-associated antigen-1/intercellular adhesion molecule-1 (LFA-1/ICAM-1) interactions. One group of cytoadhesion molecules that might be involved in the mediation of the observed inducible adhe-
signal phenomena are leukocyte \( \beta_2 \)-integrins.\(^{35} \) We therefore performed inhibition experiments and preincubated KG-1a cells with blocking MoAbs against \( \beta_1 \)-integrins (CD49d, CD49e, CD29) and \( \beta_2 \)-integrins (CD11a, CD18), ICAM-1 (CD54), LFA-3 (CD58), CD44, and CD31 before addition of MoAb QBEND10.

As shown in Table 4, we could indeed inhibit but not completely abrogate the homotypic adhesion of KG-1a cells by preincubation with the blocking CD11a MoAbs 5E6 and 6B7, as well as the CD18 MoAbs M232 and MHM23. The same was also true for the anti–ICAM-1 (CD54) MoAb RR1/1, which also significantly diminished QBEND10 MoAb-induced aggregation of KG-1a cells. MoAbs directed against \( \beta_1 \)-integrins (\( \alpha \)- and \( \beta \)-chains), LFA-3 (CD58), CD44, and CD31, all of which are expressed by KG-1a cells, did not show any inhibition of the QBEND10-induced homotypic adhesion. MoAbs to other \( \beta_2 \)-integrins (CD11b or CD11c) were not evaluated because they are not expressed on KG-1a cells.

**Table 3. Effect of Protein Kinase Inhibitors on CD34 MoAb-Induced Homotypic Cell Adhesion**

<table>
<thead>
<tr>
<th>Inhibitors</th>
<th>Adhesion Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Staurosporine</td>
<td>3+</td>
</tr>
<tr>
<td>Herbimycin A</td>
<td>0+</td>
</tr>
<tr>
<td>H7</td>
<td>3+</td>
</tr>
<tr>
<td>None</td>
<td>3+</td>
</tr>
</tbody>
</table>

KG-1a cells were preincubated with inhibitors for 30 minutes at 37°C before initiation of the adhesion assay by adding CD34 MoAb QBEND10 (final concentration 5 \( \mu \)g/mL). Aggregation was scored after 60 minutes. Identical results were obtained in three independent experiments. Inhibitors were used as follows: staurosporine (1 \( \mu \)mol/L), herbimycin A (5 \( \mu \)mol/L), and H7 (100 \( \mu \)mol/L).

...KG-1 cells might play a role and prevent KG-1 cells from marked homoaggregate formation. However, the critical involvement of additional molecule-systems cannot be excluded.

Supposing this speculation is true, it could be expected that KG-1 cells can be induced via CD34 to adhere to other ICAM-1–expressing cells. We therefore performed heterotypic adhesion experiments and mixed QBEND10-incubated KG-1 cells marked with the vital dye BCECF with unlabeled ICAM-1-positive Daudi or HL60 cells. As can be seen from Fig 5, CD34 MoAb QBEND10 led to clearcut heteroaggregate formation between KG-1 and Daudi or HL60 cells. Only minimal adhesion could be detected in the absence of QBEND10 or when negative control MoAbs were used instead of QBEND10.

**Table 4. Inhibition of QBEND10 MoAb-Induced Homotypic Adhesion by MoAbs to CD11a, the \( \beta_2 \)-Integrin \( \beta \)-Chain (CD18), and ICAM-1 (CD54)**

<table>
<thead>
<tr>
<th>Inhibiting MoAbs</th>
<th>Adhesion Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>6B7 (CD11a)</td>
<td>1+</td>
</tr>
<tr>
<td>5E6 (CD11a)</td>
<td>1+</td>
</tr>
<tr>
<td>MHM23 (CD18)</td>
<td>2+</td>
</tr>
<tr>
<td>M232 (CD18)</td>
<td>1+</td>
</tr>
<tr>
<td>RR1/1 (CD54)</td>
<td>1+</td>
</tr>
<tr>
<td>4B4 (CD29)</td>
<td>3+</td>
</tr>
<tr>
<td>HP2.1 (CD49d)</td>
<td>3+</td>
</tr>
<tr>
<td>SAM1 (CD49e)</td>
<td>3+</td>
</tr>
<tr>
<td>TS2/9 (CD58)</td>
<td>3+</td>
</tr>
<tr>
<td>185 (CD31)</td>
<td>3+</td>
</tr>
<tr>
<td>6B6 (CD44)</td>
<td>3+</td>
</tr>
<tr>
<td>VIP1 (CD71)</td>
<td>3+</td>
</tr>
<tr>
<td>VIAP (negative control)</td>
<td>3+</td>
</tr>
<tr>
<td>None</td>
<td>3+</td>
</tr>
</tbody>
</table>

KG-1a cells were preincubated with the respective MoAbs for 30 minutes at 0°C before initiation of the adhesion assay by adding CD34 MoAb QBEND10 (final concentration 5 \( \mu \)g/mL). Identical results were obtained in three independent experiments.

**DISCUSSION**

Results presented in this report show that the hematopoietic progenitor cell surface molecule CD34 has signal transducing capacity. Engagement of certain epitopes on CD34 molecules induced actin polymerization in hematopoietic cells and strongly enhanced cytoadhesiveness.

The observed enhanced cytoadhesion and aggregate formation on stimulation via CD34 seems, at least in part, to be caused by a concomitant activation of the \( \beta_2 \) integrin cytoadhesion pathway similar to the enhanced adhesion of follicular dendritic cells and B cells triggered via CD44 as reported by Koopman et al.\(^{34} \) CD34 MoAb-induced adhesion can be significantly inhibited, although not completely abolished, with blocking antibodies against binding sites of the \( \beta_2 \) integrin LFA-1 (\( \alpha_\beta \), CD11a/CD18) and its counter receptor structure ICAM-1. MoAbs directed against \( \beta_1 \)-integrins, LFA3, CD44, and CD31 did not show any inhibition of the QBEND10-induced homotypic adhesion. Homo-
typic adhesion can only be induced in ICAM-1\(^{-}\) KG-1a cells, but not in ICAM-1\(^{+}\) KG-1 cells that can be induced via CD34 to form heterotypic aggregates with ICAM-1\(^{+}\) Daudi or HL-60 cells. Activation of the \(\beta_2\) integrin pathway thus seems to play a critical role, but other not yet identified cytoadhesion molecules are probably also involved in this process.

The signaling pathways initiated by engagement of the CD34 molecule with certain CD34 antibodies are not as yet fully understood. The observation that CD34-induced adhesion induction cannot be inhibited with potent PKC inhibitors such as staurosporine\(^{31}\) and H7\(^{32}\) argues against a critical involvement of PKC activation in the signaling pathway. In line with that are also the observations that CD34 MoAb-induced adhesion induction in KG-1a cells is not accompanied by a significant increase in intracellular Ca\(^{2+}\) levels and that direct PKC activation with PMA does not induce enhanced homotypic aggregation (data not shown). However, PKC activation via phorbol ester treatment leads to adhesion of KG-1a cells to plastic, which could be inhibited by staurosporine or H7 at concentrations identical to that used in the experiments depicted in Table 3. However, a phosphorylation event seems to be important. CD34 MoAb-induced cytoadhesion could be completely inhibited by herbimycin A, a selective inhibitor of several protein tyrosine kinases.\(^{30,35}\)

The finding that the hematopoietic progenitor cell surface molecule CD34 has signal transducing capacity raises the question about the nature of potential natural ligands. Given the particular molecular structure of CD34, lectin type molecules with sugar binding activity would seem to be likely candidates.

The CD34 molecule, like the molecules CD43, CD68, lamp-1, lamp-2, and GlyCAM-1,\(^{5,10,36-39}\) has characteristic features of cell-associated lectins. Such molecules are characterized by an extended rodlike conformation\(^{40}\) that could protrude above the glycocalyx of the cell and present the numerous attached glycans to potential lectin-type counter-receptor structures. This particular configuration led Williams to the suggestion that one of the functions of hematopoietic mucins is to present carbohydrate ligands to selectins.\(^{41}\)

For the sialomucin molecules GlyCAM-1 (binding to L-selectin), lamp-1, and lamp-2 (binding to E-selectin), such sugar-lectin interactions have indeed already been shown.\(^{38,42}\) Very recent observations by Baumhueter et al\(^{43}\) show that CD34 molecules on certain endothelial cells have the capacity to bind an IgG chimera of the leukocyte surface lectin molecule L-selectin.

This high affinity binding of L-selectin-IgG chimera to CD34 molecules on capillary EC might be a particular cell type-restricted feature. When testing L-selectin-IgG chimera molecules (provided by L.A. Lasky) with strongly CD34-positive KG-1a or KG-1 cells, we were unable to detect binding (data not shown). This may suggest that the glycosylation patterns of CD34 are perhaps slightly different in different cell types. KG-1 cells may have lost the capacity to bind L-selectin or CD34 molecules on hematopoietic progenitor cells in general may interact with other ligands than do CD34 molecules on endothelial cells. In this context, it is of interest to note that the binding of all four adhesion-inducing CD34 antibodies but none of the nonstimulatory antibodies was completely abolished on treatment of CD34\(^{+}\) cells with O-sialoglycoprotease, which is known to cleave specifically the O-sialylated portions of a protein.\(^{20,44}\) The stimulatory antibodies might thus act as surrogate ligands mimicking the binding of natural counter-receptors by interfering with glycosylated regions of the CD34 molecule. Such stimulatory effects of lectin-carbohydrate interactions leading to \(\beta_2\) integrin activation have been amply documented in previous reports.\(^{45,46}\)

Considering the potential functional consequences of \(\beta_2\) integrin activation via the CD34 molecule, the selective expression of both CD34 and LFA-1 structures during hematopoietic differentiation must be envisaged. The CD34 molecule is strongly expressed on immature hematopoietic progenitor cells and shows a progressive and rapid decline in expression density with increasing hematopoietic cell differentiation.\(^{47,49}\) The regulation of CD34 expression seems to be a complex process and occurs at both transcriptional and posttranscriptional levels.\(^{11,50}\) The \(\beta_2\) integrin molecule LFA-1, on the other hand, is expressed on virtually all mature human leukocytes and the vast majority of
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