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BEN/SC1/DM-GRASP, a homophilic adhesion molecule, is required for *in vitro* myeloid colony formation by avian hemopoietic progenitors

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ABSTRACT **BEN/SC1/DM-GRASP is a membrane glycoprotein of the immunoglobulin superfamily isolated in the chick by several groups, including ours. Its expression is strictly developmentally regulated in several cell types of the nervous and hemopoietic systems and in certain epithelia. Each of these cell types expresses isoforms of BEN which differ by their level of N-glycosylation and by the presence or absence of the HNK-1 carbohydrate epitope. In the present work, the influence of glycosylation on BEN homophilic binding properties was investigated by two *in vitro* assays. First, each BEN isoform was covalently coupled to microspheres carrying different fluorescent dyes and an aggregation test was performed. We found that homophilic aggregates form indifferently between the same or different BEN isoforms, showing that glycosylation does not affect BEN homophilic binding properties. This was confirmed in the second test, where the BEN-coated microspheres bound to the neurites of BEN-expressing neurons, irrespective of the isoform considered. The transient expression of the BEN antigen on hemopoietic progenitors prompted us to see whether it might play a role in their proliferation and differentiation. When added to hemopoietic progenitor cells in an *in vitro* colony formation assay anti-BEN immunoglobulin strongly inhibited myeloid, but not erythroid, colony formation although both types of precursors express the molecule.**

The BEN molecule is a membrane protein belonging to the immunoglobulin superfamily and is transiently expressed during avian embryogenesis by a variety of cells types (1). The sequence of the cDNA encoding the BEN protein was found to be virtually identical to the sequences of the cDNAs encoding the avian molecules DM-GRASP (2) and SC1 (3). The BEN expression pattern was initially studied by means of a monoclonal antibody (mAb) produced from an immunization against the epithelium of the bursa of Fabricius (4). Besides the bursal epithelium, the BEN antigen is expressed by all peripheral neurons and, in the central nervous system, by motoneurons and some axonal tracts of the brain (5), including the cerebellar climbing fibers (6). Transient BEN expression was also found in subpopulations of hemopoietic cells. Myeloid and erythroid progenitor cells express BEN but downregulate it as differentiation proceeds (7). In the immune system, BEN is present on immature thymocytes and disappears as they acquire the phenotype of mature, quiescent $CD4^+$ or $CD8^+$ T lymphocytes. Thus, BEN was never detected on T cells in blood and posthatching spleen. Upon activation, T lymphocytes reacquire BEN at their surface (8). BEN is not expressed at any differentiation stage by cells of the B-lymphoid lineage.

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Homologs of BEN/SC1/DM-GRASP molecule have now been identified in fish (9, 10), rat (11, 12), zebrafish and mouse (13), and human (14). Homophilic adhesion properties were demonstrated for SC1/DM-GRASP (3, 15) and its human homolog (14).

BEN/DM-GRASP/SC1 is structurally related to three other molecules: MUC 18, a tumor progression marker in human melanoma (16); gicerin, a chick adhesion molecule involved in neurite outgrowth (17); and IrreC, a *Drosophila* protein involved in axonal guidance and cell death control (18). These molecules share the same arrangement of immunoglobulin domains characterized by two N-terminal variable regions followed by three constant type-2 domains. Their overall homology level is 25% along the entire extracellular portion.

Our previous studies on the biochemical characterization of the BEN protein showed that the molecules expressed in neurons, epithelia, and hemopoietic cells had differing electrophoretic mobilities depending upon their respective level of N-linked glycosylation. In particular, the neural (n-BEN) and, to a lesser extent, the hemopoietic (h-BEN) isoforms carry the HNK-1 glucidic epitope considered to be functionally important in adhesion processes (19). The protein purified from the bursal epithelium (e-BEN) is devoid of anti-HNK-1 reactivity (1).

In the work reported here, we set up an experimental system based on the use of fluorescent microspheres coupled with the purified isoforms of the BEN protein, in order to test and compare their adhesive properties. Formation of aggregates of BEN-coated beads showed that this molecule is able to mediate homophilic binding whatever its level of glycosylation and regardless of the presence or absence of the HNK-1 epitope.

In view of the general expression of the BEN glycoprotein on all types of hemopoietic progenitor cells except those engaged in the B-cell differentiation pathway, we decided to investigate a possible role for this molecule in the early steps of colony formation. Antibodies against BEN were used at various concentrations in an appropriate culture assay. BEN antibodies exerted a negative effect on all types of myeloid colony formation but did not affect the development of erythroid progenitors.

MATERIALS AND METHODS

Proteins and Antibodies. The BEN glycoprotein was immunopurified from embryonic day 13 (E13) chicken brain and from posthatching day 21 (P21) thymus and P21 bursa of Fabricius, as described (1).

Abbreviations: BFU, burst-forming unit; BSA, bovine serum albumin; CFU, colony-forming units; DRG, dorsal root ganglion; E*n*, embryonic day *n*; FACS, fluorescence-activated cell sorter; mAb, monoclonal antibody; P*n*, posthatching day *n*.

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The anti-BEN mAb is a mouse IgG1 (4). A polyclonal antibody against BEN was raised in rabbit as described (6). The IgG was purified from the polyclonal serum and Fab fragments were prepared by proteolytic digestion using the ImmunoPure Fab preparation kit (Pierce). Nonimmune rabbit IgG and the corresponding Fab fragments were prepared for use as controls.

The IgG from the mAbs HIS-C7 and 11C3, which recognize determinants present on leukocytes (20) and cells from the thrombocytic lineage (21), respectively, were also purified and used as negative controls.

Coupling of Proteins to Fluorescent Microspheres. Fluorescent polymer microspheres (Covaspheres MX reagent) with a diameter of 0.5 μm were used (Duke Scientific Corp., Palo Alto, CA). Both green (fluorescein isothiocyanate, FITC) and red (tetramethylrhodamine isothiocyanate, TRITC) fluorescent microspheres were covalently coupled to proteins according to Kuhn *et al.* (22). Before coupling, the microspheres were sonicated in a bath sonicator (Branson) for 2 min. Twenty-five microliters of beads was incubated for 1 hr at 37°C with 7 μg of purified protein—i.e., one of the BEN isoforms, hemopoietic (h), epithelial (e), or neural (n)—and two control proteins—bovine serum albumin (BSA) and IgG—in a final volume of 100 μl of phosphate-buffered saline (PBS).

The beads were washed in PBS containing 0.5% BSA and 10 mM sodium azide with sonication between washes. They were stored in the same buffer at 4°C. The concentration of Covaspheres in this stock solution was $\approx 2 \times 10^{10}$.

Covasphere Aggregation. Protein-conjugated Covaspheres were dissociated by sonication for 2 min in a bath sonicator. Beads were incubated for aggregation at 37°C during 1 hr in a final volume of 20 μl of PBS. One microliter of protein-conjugated Covaspheres was used and the concentration ratio between pairs was 1:1.

Pretreatment of one of the two Covasphere samples with Fab fragments of antibodies was performed at room temperature for 1 hr and the antibodies were removed by centrifugation ($2000 \times g$, 10 min) and by washing the pellet twice with PBS. Sonication was performed between the washes. Nonimmune and anti-BEN Fab fragments of rabbit IgG were used at 500 μg/ml. PBS without Ca^{2+} and Mg^{2+} was used.

Flow Cytometry. The interactions of protein-coated Covaspheres were analyzed on a fluorescence-activated cell sorter (FACS 440; Becton Dickinson) equipped with a 15-mW argon laser emitting at 488 nm as excitation wavelength. With classical optical filters, green fluorescence (FITC) was collected at 525 ± 10 nm and red fluorescence (TRITC) was collected at 620 ± 20 nm. All fluorescence measurements were made with a logarithmic amplifier.

Electronic compensation was used to avoid most of the spectral overlap of red and green fluorescence. The low absorption of the 488-nm laser wavelength excitation by TRITC was compensated by the intense brightness of the TRITC Covaspheres and by a stronger amplification of red versus green fluorescence.

Cell Cultures. Dorsal root ganglion (DRG) or sympathetic ganglion cells were cultured as described (23). Ganglia were dissected from chicken embryos at E11 or E12, and cells were dissociated with 0.25% trypsin for 30 min at 37°C and grown in SFRI4 medium (SFRI Laboratoire, France) supplemented with 2.5S nerve growth factor (Boehringer Mannheim) at 20 ng/ml in 35-mm dishes (Nunc). The cultures were maintained at 37°C in an atmosphere of 5% CO_2/95% air.

Cell-Binding Assays. After two washes with prewarmed Dulbecco's modified Eagle's medium (DMEM; GIBCO), the binding of green- or red-fluorescing Covaspheres (1 μl) to cultured cells in 35-mm dishes was assessed in 1 ml of DMEM for 1 hr at 37°C. Fab antibody fragments (immune and nonimmune, 500 μg/ml) were preincubated with the protein-coupled Covaspheres at room temperature for 1 hr and the spheres were washed before addition to the cultures.

The cultures were washed twice with DMEM and photographed under UV light with an inverted microscope.

To identify cells after Covasphere binding on neurons, the cultured cells in the 35-mm dishes were fixed for 45 min with 4% paraformaldehyde in 0.05 M phosphate buffer, pH 7.4. After extensive washing with PBS, the cells were treated with

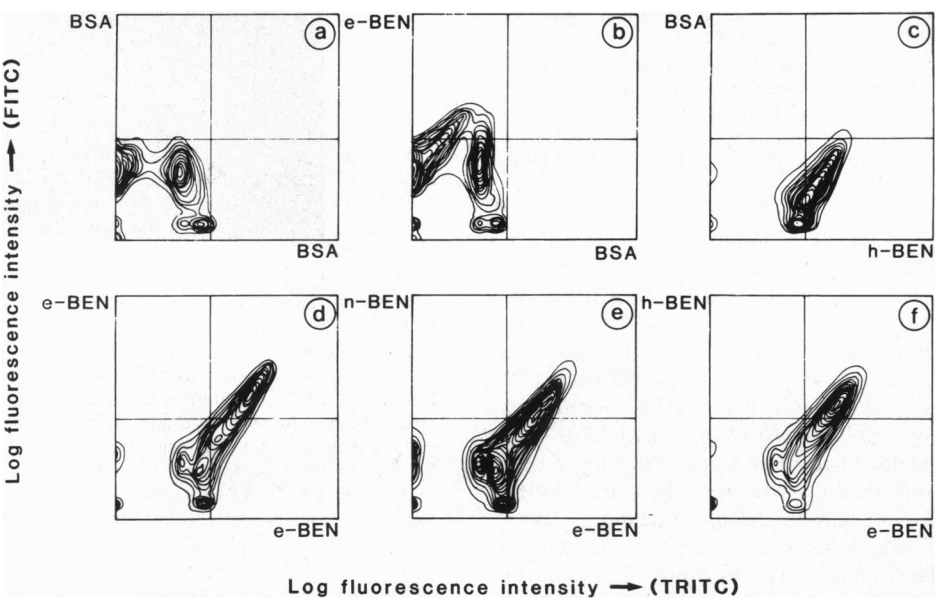

FIG. 1. Mixed-aggregate formation by Covaspheres coated with the BEN isoforms. Protein-coated Covaspheres were incubated in dual combinations with a 1:1 ratio and allowed to aggregate. The relative content of red (TRITC) or green (FITC) spheres was analyzed with a FACS. Contour plots in a two-dimensional representation of the relative fluorescence intensity are represented. (*a*) Negative control with no aggregation: BSA-conjugated FITC Covaspheres were incubated with BSA-conjugated TRITC Covaspheres. (*b* and *c*) Self-aggregation of BEN. Epithelial BEN isoform (e-BEN)-conjugated FITC-Covaspheres were incubated with BSA-conjugated TRITC-Covaspheres (*b*) or hemopoietic BEN isoform (h-BEN)-conjugated TRITC-Covaspheres were incubated with BSA-conjugated FITC-Covaspheres. (*d–f*) Mixed (red and green) aggregation when e-BEN-conjugated TRITC-Covaspheres were incubated with FITC-Covaspheres conjugated to e-BEN (*d*), neural BEN isoform (n-BEN) (*e*), or h-BEN (*f*).

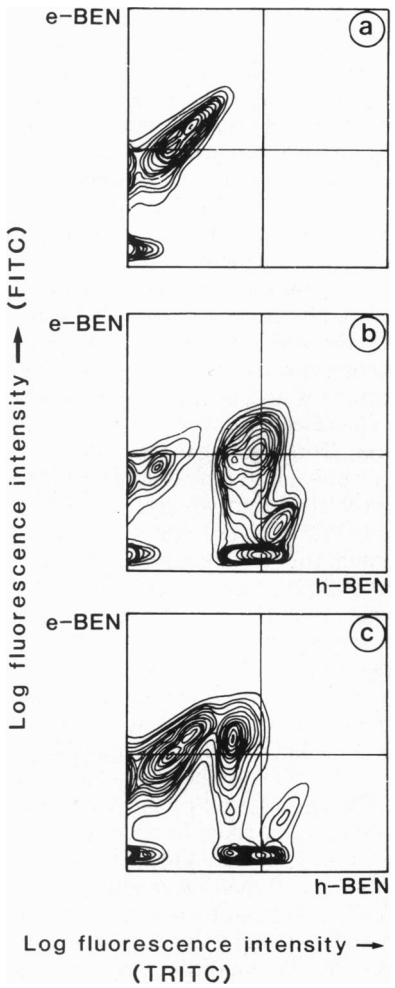

FIG. 2. Inhibition of homophilic binding of BEN isoforms. Microspheres of different colors were covalently coupled with epithelial BEN isoform (e-BEN) and incubated alone (*a*) or in dual combinations with hemopoietic BEN isoform (h-BEN), at a 1/1 ratio (*b* and *c*). (*a*) Self-aggregation of e-BEN-coated beads. (*b*) Mixed aggregates of e-BEN-coated and h-BEN-coated beads in the presence of nonimmune Fab during preincubation of h-BEN-coated beads. (*c*) Preincubation of h-BEN-coated beads with anti-BEN Fab inhibited almost completely the formation of mixed aggregates and inhibited markedly the self-aggregates of h-BEN.

0.25% Triton for 15 min. Indirect immunofluorescence staining was performed after washing in PBS, as described (7). The anti-BEN mAb was used at 1:500 dilution of ascitic fluid. Immunofluorescence staining was performed with FITC-coupled anti-IgG1 antibodies (Southern Biotechnologies) at 1:50. A rabbit polyclonal antibody against neurofilament 200 protein (Sigma) was used at 1:250 with TRITC-conjugated goat anti-rabbit immunoglobulin (Nordic) diluted 1:50.

Assay for Hemopoietic Progenitor Cells. Progenitor cells from E15–E18 chicken bone marrow were detected by their colony-forming ability in semisolid culture medium (7, 24).

Myeloid colony-forming cells were developed by addition of 3% (vol/vol) fibroblast-conditioned medium. Granulocytic and thromboblastic/erythroblastic colonies and clusters, both developed by addition of 15% E10 kidney-conditioned medium (C.C., unpublished work). Erythroid progenitor cells and burst-forming units (BFU-E) were caused to differentiate by addition of transforming growth factor α (Biomedical Technologies) at 1 ng/ml, bovine insulin (Sigma) at 10 ng/ml, and recombinant mouse erythropoietin (provided by E. Goldwasser) at 0.5 unit/ml; as described by Pain *et al.* (25).

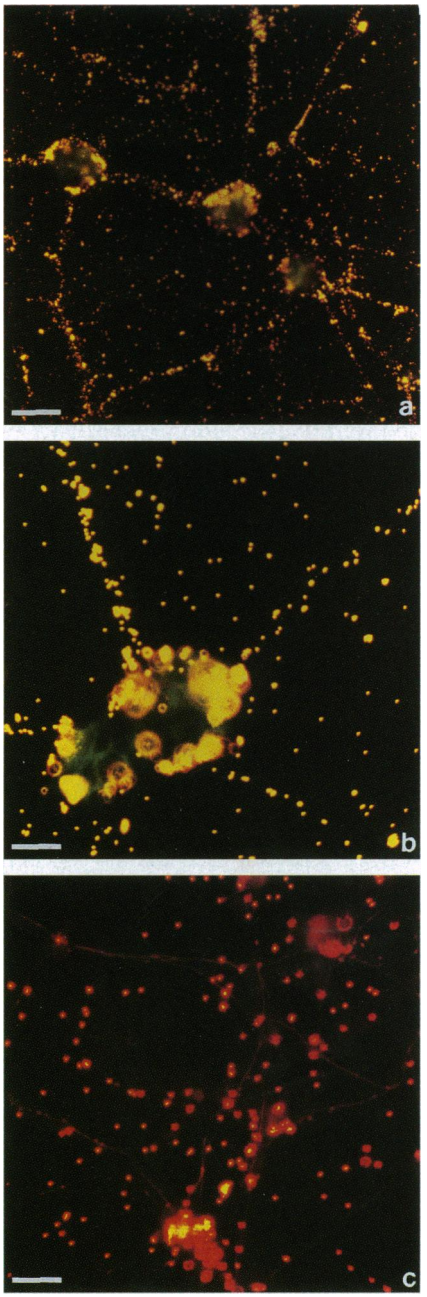

FIG. 3. Binding assay of red-fluorescing BEN-Covaspheres to neurons developed in short-term cultures from either sympathetic ganglion cells or DRG cells. (*a* and *b*) E11 sympathetic ganglion cells cultured for 48 hr were incubated with e-BEN-conjugated TRITC-Covaspheres. Cells expressing BEN were identified after microspheres binding by FITC immunofluorescence using the anti-BEN mAb. A strong BEN expression at the intercellular contacts of the cell bodies can be seen in *b*. Note that nonneuronal cells are neither BEN^+ nor binding to the Covaspheres. (*c*) E11 DRG incubated with n-BEN-conjugated TRITC-Covaspheres. Double immunofluorescence staining was performed thereafter, with the anti-BEN mAb revealed with an anti-mouse IgG1-FITC conjugate and with an antibody against neurofilaments revealed with an anti-rabbit-TRITC conjugate. Neurons, which expressed neurofilaments, were BEN-negative and did not bind BEN-coated beads. [Bars = 75 μm (*a*) or 20 μm (*b* and *c*).]

The cultures were subjected to morphological and quantitative analysis after May–Grünwald–Giemsa staining. In some cases, the slides were stained with a DNA-intercalating fluorescent dye (Hoechst 33258) and observed with a fluorescence microscope.

Table 1. Effect of anti-BEN antibodies on *in vitro* growth of bone marrow myeloid progenitors

Antibody	Conc., μg/ml	No. of colonies and clusters per plate* CFU-M	CFU-G	CFU-GM
		Experiment 1		
None	—	20 ± 4	6.5 ± 1.5	3.5 ± 0.5
Anti-BEN	300	15 ± 1 (25%)†	2 ± 0 (69%)†	1.5 ± 1.5
	400	5 ± 1 (75%)†	0 (100%)	0
		Experiment 2		
None	—		16.5 ± 1.5	3.5 ± 0.5
Anti-BEN	300		6 ± 1 (63%)†	0
	400		4 ± 0 (76%)†	0
Nonimmune Fab	80		16 ± 3	0
Anti-BEN Fab	60		12 ± 1 (25%)†	1.5 ± 0.5†
	80		6.5 ± 0.5 (60%)†	0

Experiments used in this table are representative of a larger series (10 experiments) of similar tests.

*Means ± SEM of duplicate cultures seeded with 3×10^3 and 6×10^3 bone marrow cells for experiments 1 and 2, respectively. The percent inhibition of colony formation by anti-BEN immunoglobulin in comparison with nonimmune immunoglobulin or no immunoglobulin is given in parentheses. CFU, colony-forming units; M, macrophage; G, granulocyte; GM, granulocyte/macrophage.

†Colonies altered in size and/or morphology.

RESULTS

Homophilic Adhesion. *Flow cytometry studies.* The ability of BEN to mediate homophilic cell adhesion was demonstrated in aggregation assays using fluorescent polystyrene beads (Covaspheres) and analyzed by flow cytometry.

To test whether the sugar moiety of the BEN molecule is involved in cell adhesion, each of the three differently glycosylated isoforms of the protein was coupled to FITC-Covaspheres and tested for aggregation with another BEN isoform conjugated to TRITC-Covaspheres. The mutual affinity of the conjugated microspheres was analyzed by measuring their ability to form self or mixed aggregates (Fig. 1).

Covaspheres coated with BSA (Fig. 1*a*) or IgG (data not shown) did not have tendency to self-aggregate. Their fluorescence emission for both FITC and TRITC occurred at the intensity of single or doublet beads. When incubated together, Covaspheres conjugated with BSA and linked either to FITC or to TRITC did not form mixed aggregates, as expected.

As shown for epithelial BEN (e-BEN) and hemopoietic BEN (h-BEN), each BEN isoform is able to form self-aggregates (Fig. 1 *b* and *c*). Therefore, this experimental system appeared suitable to study the role of glycosylation in homophilic interactions.

Table 2. Specific inhibition of CFU-G by anti-BEN antibody

Antibody	Conc., μg/ml	No. of colonies and clusters per plate* Erythrocytes	Thromboblasts/ erythroblasts	Granulocytes
		Experiment 1		
None	—	88.5 ± 0.5	14 ± 1	0
Nonimmune	400	81 ± 8	12 ± 5	0
Anti-BEN	400	94 ± 18	27 ± 5	0
Nonimmune Fab	80	76 ± 10	24.5 ± 7.5	0
Anti-BEN Fab	80	75.5 ± 6.5	16.5 ± 0.5	0
		Experiment 2		
None	—	0	480 ± 14	350 ± 14
Anti-BEN	400	0	428 ± 2	2 ± 1
Anti-11C3	400	0	276 ± 66	345 ± 47

For experiment 1, cultures were seeded with 6×10^3 bone marrow cells from E15 embryos; medium contained serum and transforming growth factor α. For experiment 2, cultures were seeded with 12×10^3 bone marrow cells from E14 embryos; medium was serum-free but contained kidney conditioned medium.

*Means ± SEM of duplicate cultures.

Conjugated beads incubated in combinations between the three isoforms formed mixed aggregates, as shown for two of them in Fig. 1 *e* and *f*. When n-BEN-, and e-BEN-conjugated beads of different fluorescent colors were mixed, incubated, and analyzed by FACS, almost all the aggregates were mixed even if some beads remained single or in doublets. Only mixed aggregates were also detected from other dual BEN isoform combinations such as h-BEN and n-BEN. The FACS profiles were similar to those obtained when the same BEN isoform—for instance, e-BEN (Fig. 1*d*)—was conjugated to FITC-Covaspheres and TRITC-Covaspheres which were then coincubated.

The specificity of the interactions observed between the different BEN isoforms was tested as follows. Covaspheres coated with a given BEN were first incubated with either nonimmune or anti-BEN Fab fragments. Then they were mixed with Covaspheres coated with another BEN isoform. The results are shown in Fig. 2 for e-BEN and h-BEN Covaspheres mixed together. As already shown, e-BEN-conjugated FITC-Covaspheres incubated alone formed aggregates (Fig. 2*a*). Incubation of h-BEN-conjugated TRITC-Covaspheres with nonimmune Fab fragments prior to their addition to e-BEN-Covaspheres altered neither the formation of h-BEN self-aggregates nor the formation of mixed aggregates. Even when e-BEN self-aggregates were obtained, a large amount of the e-BEN-coated Covaspheres was included in the mixed aggregates formed with h-BEN-coated Covaspheres (Fig. 2*b*). Preincubation of the h-BEN-Covaspheres with anti-BEN Fab fragments prevented the formation of h-BEN self-aggregates as well as mixed aggregates (Fig. 2*c*).

This demonstrates that the interactions observed between BEN isoforms are specific, since monovalent antibodies to BEN selectively suppress homophilic BEN binding. These interactions are independent of the level of glycosylation which characterizes the different BEN isoforms and of the presence or absence of the HNK-1 epitope, which is found on nervous and hemopoietic cells but not on bursal epithelial cells (1).

Binding of BEN isoforms on cultured cells. We then investigated whether the microspheres coated with various BEN isoforms bound equally to BEN-positive cells in culture. Sympathetic neurons, which intensely express the BEN molecule from E10 onward, were cultured for 2 days. In parallel, E11 DRG cells were chosen as negative controls, since BEN is present in DRG only transiently, from E3 to E10 (4).

Cultures of both types of neurons were exposed to Covaspheres coated with one of the three BEN isoforms. e-BEN-conjugated Covaspheres bound to cell bodies and neurites of dissociated E11 sympathetic ganglia (Fig. 3 *a* and *b*). No binding occurred between n-BEN-coated beads and E11 DRG neurons (Fig. 3*c*). Similar results were obtained when h-BEN- and n-BEN-coated Covaspheres were applied to the neuronal cultures under the same conditions. Preincubation of the Covaspheres with anti-BEN Fab fragments completely prevented their binding to neurites and cell bodies. In contrast, preincubation of BEN isoform-coated Covaspheres with nonimmune Fab fragments had no effect on binding with BEN-coated Covaspheres (data not shown).

These results show that BEN binding on neurons is homophilic and that the differences in glycosylation of each BEN isoform does not modify the homophilic interactions of the molecule.

***In Vitro* Studies of the Role of BEN in Hemopoiesis.** We have previously shown that bone marrow hemopoietic progenitors express the BEN molecule (7). Therefore to assess a possible functional role of BEN in colony formation, we treated hemopoietic cells with anti-BEN antibody and then assayed their capacity to form colonies in semisolid cultures. Bone marrow mononuclear cells were exposed to the anti-BEN antibody or to derived Fab fragments. The number of macrophage (M), granulocyte (G), and granulocyte/macrophage

(GM) colonies was markedly reduced in the presence of the antibody. Two representative experiments among 10 are shown in Table 1. The level of inhibition was correlated with the concentration of immunoglobulin or Fab fragments of anti-BEN polyclonal antibody. Moreover, the size and morphology of the colonies were altered in the presence of suboptimal doses of antibody: the Hoechst DNA staining showed typical apoptotic features, with condensed and fragmented nuclei in granulocytic and monocytic colonies (data not shown). The addition of the same amount of an antibody directed against HIS-C7, an antigen expressed by all leukocytes (20), had no effect on the differentiation of myeloid progenitors under identical conditions. In strong contrast, anti-BEN treatment did not affect erythroid colony formation deriving from BFU-E progenitors, even at an immunoglobulin concentration which led to total inhibition of myeloid progenitor cells (Table 2; representative data are shown from one of six experiments performed).

This was also demonstrated under experimental conditions allowing the simultaneous formation of both granulocytic and erythroblastic/thromboblastic colonies and clusters. These progenitors proliferated and differentiated in response to growth factors contained in kidney conditioned medium, as recently found (C.C., unpublished work). Anti-BEN IgG (400 μg/ml) totally inhibited the formation of granulocyte colonies and clusters and did not affect that of thromboblast/erythroblast clusters. Such an inhibitory effect was not obtained with an irrelevant antibody (11C3; ref. 21) used at the same concentration (Table 2). Terminal differentiation in either thrombocytes or erythrocytes did not occur under these culture conditions, making it difficult to ascertain which lineage was involved, erythroid or thrombocytic. Embryonic erythroblasts and thromboblasts are undistinguishable after May–Grünwald–Giemsa staining (26) and are likely to be derived from a common precursor cell (27). This is in line with our observation that thromboblastic/erythrocytic colonies were not inhibited by anti-BEN antibodies. Therefore, the inhibition by anti-BEN antibodies is restricted to the myeloid lineage.

DISCUSSION

The BEN molecule, also designated as DM-GRASP or SC1, is expressed by several cell types during development, according to a highly controlled spatial and temporal pattern. In the nervous system, several observations point to a role of BEN in neuritic outgrowth and guidance as well as in homophilic adhesion processes involved in cell–cell recognition (see ref. 28 for review). In the hemopoietic system, BEN expression is also confined to precise developmental stages of the erythroid, myeloid, and T-lymphocyte differentiation pathway and excluded in the mature forms of these cell lineages.

The BEN proteins purified from neural, epithelial, and hemopoietic sources exhibit different levels of N-linked glycosylation (1). In particular, the HNK-1 carbohydrate epitope is found on the brain and thymus isoforms but not on the bursal isoform.

By using fluorescent BEN-coated Covaspheres, we demonstrated that each of the three BEN isoforms tested in an aggregation assay exhibited homophilic binding properties. In addition, when beads coated with different BEN-isoforms were mixed, they always formed coaggregates, thus showing that glycosylation does not confer a tissue-type specificity to the homophilic binding mediated by this molecule. It was then important to determine whether homophilic binding can occur between BEN coated on Covaspheres and the molecule embedded in the physiological environment of a cell membrane. Each BEN isoform conjugated to Covaspheres adhered to the neurites of cultured E11 sympathetic neurons, which strongly express BEN. As in the Covasphere assay, binding of the beads with the neurons was inhibited by their preincubation with anti-BEN Fab fragments. E11 DRG neurites, which do not express BEN, did not bind any BEN isoform-conjugated beads. These observations show that BEN mediates homophilic adhesion independently of the level of glycosylation.

The presence of BEN on neurons during axonal growth and the homophilic adhesion property of the molecule suggest that it may have a role in neurite fasciculation (1). Expression of this glycoprotein on all hemopoietic progenitors except for those engaged in the B-cell differentiation pathway (7, 8) raises the problem of its function in hemopoiesis.

Separation by FACS of bone marrow cells into BEN^+ and BEN^- populations showed that all CFU (M, G, and GM) as well as BFU-E and thrombocytic progenitors were found within the BEN^+ population. To determine whether BEN has a role in regulating chicken hemopoiesis, progenitor cells were treated with anti-BEN antibodies and assayed for colony formation in the presence of growth factors supporting the differentiation and proliferation of myeloid or erythroid progenitors. The results presented here provide evidence that under conditions allowing the simultaneous development of granulocytes and thromboblastic/erythroblastic colonies, the inhibition by anti-BEN antibodies was always restricted to myeloid progenitors. Therefore, BEN is required for growth of all types of chicken myeloid progenitor cells (CFU-G, CFU-M, and CFU-GM) but does not interfere with that of erythroid (BFU-E) and thrombocytic precursors in spite of the fact that they do express BEN during the early stages of colony formation. It is generally accepted that erythroblasts and thromboblasts are derived from a common precursor (27), a cell which therefore does not require BEN function for further development. One can propose that by inhibiting the homophilic binding of BEN molecules carried by the progeny of myeloid progenitors the anti-BEN antibody prevents cell–cell contacts which are necessary for further cell proliferation and differentiation to occur.

That the BEN-blocking antibody does not impair erythroid colony formation indicates that the requirements, in term of cell contacts, are different for the myeloid and erythroid precursors.

Whether the role of BEN in myeloid colony formation is restricted to cell–cell adhesion or whether it is also involved in signal transduction remains a question. The possibility that binding of the antibody delivers a negative signal to these precursors cannot be ruled out. This might imply that BEN would also bind an alien receptor in a heterophilic manner. This receptor would then be present on myeloid but not erythroid progenitors. This molecule could function either as a BEN coreceptor or through an independent heterophilic interaction. Even if the functional studies presented herein show that BEN exhibits homophilic adhesion, this does not exclude the possibility that heterophilic interactions might also occur. For the hemopoietic cells, a heterophilic binding might take place with CD6. It was recently found that the human homolog of BEN, ALCAM, is a CD6 ligand (14).

Whatever the mechanism involved, it is clear that in our experiments, the anti-BEN antibody blocked a positive signal necessary for myeloid colony development. Under the effect of the antibodies the myeloid progenitors went through one or two cell divisions and then died.

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