

CHARACTERIZATION OF THE LYMPHOCYTE MEMBRANE
RECEPTOR FOR FACTOR H (β 1H-GLOBULIN) WITH
AN ANTIBODY TO ANTI-FACTOR H IDIOTYPE*

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Factor H (β 1H globulin),¹ an important control protein of the alternative pathway of complement (C) activation, is recognized as having three characteristic functions: (a) acceleration of the decay dissociation of the C3 convertase (C3b,Bb) by binding to C3b and displacing activated factor B (Bb) (1); (b) function as an essential cofactor for the cleavage inactivation of fluid-phase C3b by factor I (C3b-inactivator) (2); and (c) potentiation of the factor I cleavage of bound C3b (1). Factor H also binds to B lymphocyte membrane receptors that are specific for H and triggers the B cells to release endogenous factor I (3). In addition, Hammann and co-workers (4) have recently reported that purified H triggers lymphocyte blastogenesis.

In the present study, an antibody directed to the binding site (idiotype) of anti-H was used to characterize the structure of B cell membrane H receptors. This strategy for receptor characterization was first described by Sege and Peterson (5), who demonstrated that an antibody to the idiotype of anti-retinol-binding protein (RBP) reacted with prealbumin, thus identifying prealbumin as the probable carrier ("receptor") for RBP in the blood. The basis for applying this approach to H receptor characterization was the hypothesis that the binding site of H receptors might be structurally similar to the idiotype of anti-H antibody and, thus, that antibody to the idiotype of anti-H might react with membrane H receptors. Accordingly, an anti-H idiotype serum was prepared by injecting rabbits with purified goat F(ab')₂ anti-human H. Several lines of evidence indicated that one of the rabbit-anti-goat-anti-human H anti-idiotype antibodies ($\alpha\alpha$ H) isolated from this antiserum was specific for human B cell H receptors. Both $\alpha\alpha$ H immunoprecipitation and H-agarose affinity chromatography were then used to characterize the molecular weight of the H receptor by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE).

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¹ *Abbreviations used in this paper:* $\alpha\alpha$ H, anti-anti-H, rabbit antibody specific for the idiotype of goat-anti-human factor H; B, factor B; BDV, 1% BSA (bovine serum albumin) and 3.2% dextrose in 35 mM veronal buffer, pH 7.2, 6 mS at 22°C; BDVEA, BDV containing 20 mM EDTA and 0.2% sodium azide; C, complement; C3b, hemolytically active fragment of C3 resulting from C activation; C3c and C3d, fragments resulting from proteolysis of iC3b; CR₁, C receptor type one, C4b-C3b receptor; CR₂, C receptor type two, C3d receptor; CR₃, C receptor type three, iC3b receptor; D, factor D; E, sheep erythrocytes; EC3b, E coated with C3b; EC3d, E coated with C3d; GVB, gelatin veronal buffer; H, factor H (β 1H globulin); iC3b, inactivated C3b resulting from cleavage of C3b by factor I (C3b-inactivator); I, factor I (C3b-inactivator); Mg-GVB, GVB containing 5 mM MgCl₂; NF, C3 nephritic factor; NP-40, Nonidet P-40; P, properdin; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis.

Materials and Methods

Lymphoid Cells. The B lymphoblastoid cell lines known as Raji and BF, and the HSB T lymphoblastoid line were maintained in RPM1 1640 media supplemented with 10% heat-inactivated fetal calf serum and antibiotics.

C Components and Sheep Erythrocyte-C3 Complexes (EC3). Human C3, factor B(B), factor H(H), and factor I(I) were purified from fresh plasma, as previously described (3). Factor D (D) and nephritic factor (NF) were purified as described by LeSavre et al. (6) and Schreiber et al. (7). EC3b, containing $1.5\text{--}2.5 \times 10^4$ molecules of C3b per cell, were prepared by addition of C3 to sheep E, containing the NF-stabilized C3-convertase (8), and the amount of resulting bound C3b was quantitated by measuring the uptake of [^{125}I]monoclonal-anti-C3 (Bethesda Research Laboratories, Gaithersburg, MD) onto a small sample of the EC3b. A portion of the EC3b was converted to EC3bi and EC3d (3).

Production of Anti-H and $\alpha\alpha\text{H}$. A goat was immunized intramuscularly with weekly injections of 50 μg of H emulsified in Freund's complete adjuvant, and the plasma was collected by plasmaphoresis (immunization and plasmaphoresis performed by Dr. Brian F. Tack, Harvard Medical School, Boston, MA). The goat immune plasma was found to be specific for H by Ouchterlony and immunoelectrophoretic analysis with human plasma and various purified C proteins. The IgG fraction of the plasma was isolated by column chromatography on DEAE-Sephacel (Pharmacia Fine Chemicals, Div. of Pharmacia Inc., Piscataway, NJ) in 0.01 M phosphate buffer, pH 8.0, and then the F(ab')₂ fragment was prepared by trypsin digestion (9) and column chromatography on Sephadex G-150 (Pharmacia Fine Chemicals). The various different H-specific idiotypes of the F(ab')₂ anti-H were then purified by absorption and 4 M guanidine elution from agarose containing 10 mg of bound H per ml of gel (10). Two rabbits were immunized weekly at two intramuscular sites with 100 μg of the purified goat F(ab')₂ anti-human H emulsified in Freund's complete adjuvant over a period of 3 mo. Nonimmune sera was obtained before immunization, and then immune sera were collected weekly after 6 wk of immunization. After isolation from the rabbit sera of both the nonimmune IgG and the IgG $\alpha\alpha\text{H}$ on DEAE-Sephacel in 0.0175 M phosphate buffer, pH 7.0, antibodies in the $\alpha\alpha\text{H}$ directed to common goat IgG determinants were removed by absorption with agarose conjugated (10) to the nonimmune goat IgG obtained by absorption of the goat IgG anti-H with H-agarose. A portion of the absorbed $\alpha\alpha\text{H}$ was either labeled with ^{125}I using chloramine T (11), resulting in a specific activity of 6.2×10^4 cpm/ μg or, alternatively, was purified by absorption and 4 M guanidine elution from either goat anti-H-agarose or C3-agarose (10 mg IgG anti-H or C3 per ml of gel) and then labeled with ^{125}I (11) to a specific activity of $4\text{--}6 \times 10^4$ cpm/ μg .

Preparation of Antibodies Specific for CR₁, CR₂, C3b, and C3c. Antibodies to CR₁, CR₂, and C3c were prepared in rabbits by immunization with the purified protein antigens (12–14). Anti-C3b (kindly provided by Dr. Brian F. Tack, Harvard Medical School) was prepared by immunization of sheep with C3b that was generated by elastase digestion of C3 and column chromatography on Sephadex G-200 (Pharmacia Fine Chemicals). The F(ab')₂, Fab', or Fab fragments of these antibodies were prepared by digestion with pepsin or papain (15, 16).

Assay for Binding of $\alpha\alpha\text{H}$ to B Cells and C3 Fragments. 100 μl of Raji, HSB, or BF cells at 4×10^6 cells/ml, or alternatively E, EC3b, or EC3d at 1×10^8 cells/ml, in 0.01 M phosphate-buffered saline, pH 7.5, were incubated for 15 min at 37°C with 25- μl volumes of increasing concentrations of [^{125}I] $\alpha\alpha\text{H}$. Each concentration of [^{125}I] $\alpha\alpha\text{H}$ was tested in duplicate, and controls included ^{125}I -nonimmune rabbit IgG, or [^{125}I] $\alpha\alpha\text{H}$ in the presence of excess unlabeled $\alpha\alpha\text{H}$ or 1.0 mg/ml H. The quantity of bound ligand was determined after centrifugation of the cells in 100 μl of suspension through 750 μl of oil at 8,000 g for 2 min (17).

Assay for Inhibition of H Binding to B Cells and EC3b by $\alpha\alpha\text{H}$. Purified H was labeled with ^3H by reductive methylation (18) and had a specific activity of 3.6×10^6 dpm/ μg . The specific binding of [^3H]H to Raji cells, BF cells, or EC3b was measured as previously described (3, 17) after treatment of the cells with either unlabeled H, $\alpha\alpha\text{H}$, or Fab anti-C3c.

Assay for B Cell Release of Factor I. The cell-free supernatants of Raji B cells incubated with either BDV buffer (1% bovine serum albumin (BSA) 3.2% dextrose, in 35 mM veronal buffer, pH 7.2, 6 mS at 22°C) or H, $\alpha\alpha\text{H}$, and Fab anti-C3c, each diluted in BDV, were analyzed for their ability to convert Raji rosette-negative EC3b into Raji rosette-positive EC3bi (3). The

specificity of this assay for I was confirmed by demonstrating that the rosette-generating activity of the cell supernatants was inhibited completely by anti-I (3).

Assay for the Factor I Cofactor Activity of Factor H and $\alpha\alpha$ H. Cleavage of ^3H -labeled fluid-phase C3b (3) into iC3b was detected by SDS-PAGE (19), fluorography (3), and laser densitometric scanning of the x-ray film bands. 2.8 μg of [^3H]C3b was treated with 1 μg of I and 10 μg of either H or $\alpha\alpha$ H in 50 μl of 30 mM NaCl in 35 mM veronal buffer, pH 7.2, for 60 min at 37°C. The potentiation of I cleavage of bound C3b by H or $\alpha\alpha$ H was assessed by treating EC3b for 30 min at 37°C with H or $\alpha\alpha$ H and an amount of I that by itself produced only minimal amounts of bound iC3b. The formation of increased amounts of iC3b on EC3b was detected either by SDS-PAGE and fluorography analysis of EC3b prepared with [^3H]C3 (3) or by generation of the ability of the treated EC3b to form Raji rosettes (3). Controls included treatment of [^3H]C3b or EC3b[^3H] with H, $\alpha\alpha$ H, or I individually.

Molecular Weight Analysis of B Cell Proteins Bound by Either $\alpha\alpha$ H or H. B cell proteins were intrinsically labeled by overnight culture of Raji or BF cells in media containing [^3H]leucine, as previously described (3). For analysis of the B cell proteins reactive with $\alpha\alpha$ H, the washed cells were solubilized with nonionic detergent (3). After removal of insoluble material by ultracentrifugation (3), the soluble B cell proteins from 5×10^7 cells in 0.5 ml of buffer (3) were incubated with 10 μg of either purified $\alpha\alpha$ H or nonimmune rabbit IgG for 16 h at 4°C. The rabbit IgG and any immune complexes were then collected by centrifugation after incubation for 4 h at 4°C with 10 μl of agarose-anti-rabbit IgG (Miles Laboratories, Inc., Research Products Div., Elkhart, IN). To remove nonspecifically bound cell proteins, the agarose-bound complexes were washed four times with ice-cold phosphate-buffered saline containing 1% Nonidet P40 (NP-40) (Particle Data Inc., Elmhurst, IL), 0.5% deoxycholate, 20% sucrose, 2 mM phenylmethylsulfonyl fluoride, 50 $\mu\text{g}/\text{ml}$ soybean trypsin inhibitor, 25 mM benzamidine, 50 mM epsilon amino caproic acid (Sigma Chemical Co., St. Louis, MO), and 10 mM EDTA. The complexes were eluted from the agarose by incubation for 5 min at 100°C with 2% SDS, either with or without 0.1 M dithiothreitol, and electrophoresed in 7% polyacrylamide gel slabs (19). The gel tracks were then sliced into 2-mm segments that were solubilized by overnight incubation in 30% H_2O_2 at 56°C and counted in OCS scintillation fluid (Amersham Corp., Arlington Heights, IL). The molecular weight of radioactive protein bands was determined from their mobility relative to known molecular weight markers (Bio-Rad Laboratories, Richmond, CA) electrophoresed in parallel gel tracks.

For molecular weight analysis of the B cell proteins that had H-binding (H receptor) activity, the spent culture media from [^3H]leucine intrinsically labeled Raji cells was cycled two times through a column containing 2 ml of either H-agarose (same as above) or control BSA-agarose (10 mg BSA per ml of agarose) (10) in 0.01 M phosphate-buffered saline, pH 7.5, with protease inhibitors (same as above). After a wash of the columns with 0.5 M NaCl in 0.1 M phosphate buffer, pH 7.5, the bound material was eluted with a mixture of 0.5% NP-40 and 0.5 M NaCl in 0.1 M phosphate buffer, pH 7.5. The eluate was dialyzed against 62.5 mM Tris/HCl, pH 6.8, concentrated to 0.5 ml in the dialysis bag by dehydration with polyethylene glycol powder, treated with 2% SDS either with or without 0.1 M dithiothreitol for 5 min at 100°C, and electrophoresed in 7% polyacrylamide gel slabs that were analyzed for radioactivity and molecular weight as above.

Results

Characteristics of $\alpha\alpha$ H. After absorption with nonimmune goat IgG-agarose, the rabbit IgG fraction of $\alpha\alpha$ H was labeled with ^{125}I and analyzed for binding to lymphocytes and EC3 (Table I). Approximately 10-fold more [^{125}I] $\alpha\alpha$ H was bound to Raji and BF B-type lymphoblastoid cells and to EC3b than was bound to HSB T-type lymphoblastoid cells, sheep E, or EC3d. By contrast, the labeled nonimmune rabbit IgG, obtained before immunization, was not bound selectively by any cell type. This absence of binding of nonimmune IgG indicated that Fc receptors were probably not a factor in the uptake of $\alpha\alpha$ H by lymphoid cells. The specificity of the binding activity was further demonstrated by the finding that excess unlabeled $\alpha\alpha$ H blocked

TABLE I
Binding of ^{125}I -labeled $\alpha\alpha\text{H}$ to Lymphocytes and EC3

Cell type	^{125}I $\alpha\alpha\text{H}$ cpm bound in presence of			^{125}I -nonimmune IgG cpm bound in buffer
	Buffer	Excess unlabeled $\alpha\alpha\text{H}$	1.0 mg/ml factor H	
B cell lines				
Raji	1,020	80	75	95
BF	1,565	120	130	150
T cell line				
HSB	125	110	105	150
E	360	295	299	306
EC3b	3,250	400	395	340
EC3d	290	250	265	275

TABLE II
Inhibition of the Binding of ^3H -labeled Factor H (^3H H) to B Cells and EC3b by $\alpha\alpha\text{H}$

Pretreatment of cells	^3H H cpm bound		
	Raji	BF	EC3b
Buffer	6,555	3,150	8,250
$\alpha\alpha\text{H}^*$	500	325	2,501
1,000-fold excess unlabeled H	456	134	2,457
Sheep IgG-anti-C3b	6,600	3,045	ND \ddagger

* Purified by absorption and elution from C3-agarose.

\ddagger Not done because anti-C3b agglutinated the EC3b.

the uptake of ^{125}I $\alpha\alpha\text{H}$. Thus, the $\alpha\alpha\text{H}$ had a binding activity that was similar to factor H in that both $\alpha\alpha\text{H}$ and H bound to B cells and to EC3b. In addition, a common binding site for H and $\alpha\alpha\text{H}$ was suggested by the finding that B cells and EC3b did not bind ^{125}I $\alpha\alpha\text{H}$ in the presence of 1.0 mg/ml of factor H (Table I).

To determine whether the $\alpha\alpha\text{H}$ contained two distinct antibodies that were specific for either anti-H (anti-idiotypic) or for C3b (anti-C3b), 200 mg of the $\alpha\alpha\text{H}$ was absorbed in succession with nonimmune goat IgG-agarose and either goat-anti-H-agarose or C3-agarose. Samples of the absorbed $\alpha\alpha\text{H}$ were then labeled with ^{125}I and analyzed for cell-binding activity. Absorption with goat-anti-H-agarose removed all B cell- and EC3b-specific binding activity, and, likewise, absorption with C3-agarose removed all B cell-specific binding activity along with EC3b-binding activity. Only 1 mg of bound IgG was recovered after 4 M guanidine elution of either the anti-H-agarose or the C3-agarose. Finally, when the two different eluted IgG fractions were labeled with ^{125}I , both bound to B cells and to EC3b in an apparently identical manner, and, in addition, the antibody eluted from C3-agarose bound to goat-anti-H-agarose (data not shown). It was concluded that probably a single anti-idiotypic antibody contained in the $\alpha\alpha\text{H}$ reacted with both B cells and C3b.

To determine whether $\alpha\alpha\text{H}$ blocked the binding of H to B cell H receptors, B cells were treated with either pure $\alpha\alpha\text{H}$ or H and then tested for their ability to bind ^3H -labeled H (Table II). Both the $\alpha\alpha\text{H}$ (100 $\mu\text{g}/\text{ml}$) and a 1,000-fold molar excess of

unlabeled H blocked the uptake of [^3H]H onto either Raji or BF cells. In addition, $\alpha\alpha\text{H}$ also blocked the uptake of [^3H]H onto EC3b. Because of this finding, a sheep IgG anti-C3b was compared with the $\alpha\alpha\text{H}$ for its ability to block the uptake of [^3H]H onto B cells. Treatment of B cells with 10 mg/ml of the sheep anti-C3b, which had an EC3b agglutinating titer of 1,600, had no effect on the uptake of [^3H]H onto membrane H receptors (Table II).

Factor H-like Activity of $\alpha\alpha\text{H}$. Because the purified $\alpha\alpha\text{H}$ resembled factor H in its binding specificity for C3b and B cell H receptors, $\alpha\alpha\text{H}$ was examined for its ability to substitute for factor H in functions that are characteristic of factor H. Purified $\alpha\alpha\text{H}$ was found to function with factor I as a cofactor for cleavage of fluid-phase C3b and as a potentiator for cleavage of bound C3b (Fig. 1 and Table III). However, on a weight ratio comparison, C3-agarose affinity-purified $\alpha\alpha\text{H}$ had only 0.1% of the activity of purified factor H. With both fluid-phase [^3H]C3b (Fig. 1) and EC3b[^3H] (data not shown), only small amounts of iC3b were detected by SDS-PAGE analysis after $\alpha\alpha\text{H}$ and I treatment. When H was used rather than $\alpha\alpha\text{H}$, I treatment resulted in complete conversion of C3b to iC3b (Fig. 1). The factor H-like activity of the $\alpha\alpha\text{H}$ with I and bound C3b was more easily demonstrated by the Raji rosette test that is sensitive to very small amounts of bound iC3b (3) (Table III).

Because the binding of factor H to B cell H receptors had previously been shown to stimulate the release of B cell endogenous factor I (3), $\alpha\alpha\text{H}$ was tested for its ability to trigger I release from Raji B cells (Table IV). After treatment of B cells with either purified $\alpha\alpha\text{H}$ or H, the cells were pelleted by centrifugation, and the harvested cell-

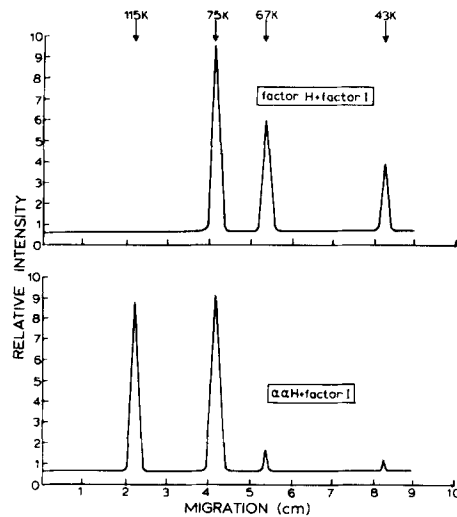


FIG. 1. Factor I cofactor activity of $\alpha\alpha\text{H}$ vs. factor H in the cleavage of fluid-phase [^3H]C3b. After incubation of [^3H]C3b for 60 min at 37°C with either H plus I or $\alpha\alpha\text{H}$ plus I, samples were analyzed by SDS-PAGE, fluorography, and laser densitometric scanning of x-ray film bands. The molecular weight of each gel band was determined from its mobility relative to known molecular weight markers run in parallel gel tracks. [^3H]C3b treated with H plus I (upper panel) was fully converted into iC3b, as indicated by the complete absence of intact C3b α -chains (115,000 M_r) and the appearance of the two characteristic iC3b α -chain fragments (67,000 and 43,000 M_r). By contrast, treatment of [^3H]C3b with $\alpha\alpha\text{H}$ plus I (lower panel) resulted in conversion of only small amounts of C3b into iC3b. No iC3b was detected in controls in which [^3H]C3b was treated with H, $\alpha\alpha\text{H}$, or I, individually (data not shown).

TABLE III
Potential of Factor I Cleavage of Bound C3b into iC3b by Factor H or $\alpha\alpha$ H

Treatment of EC3b 10 min, 37°C	Raji rosettes with treated EC3b*
	%
Buffer	0
Factor H‡	0
Limiting factor I	20
Limiting factor I and factor H	99
$\alpha\alpha$ H§	0
Limiting factor I and $\alpha\alpha$ H	90
Limiting factor I and nonimmune IgG	18

* Rosette assay performed in BDVEA buffer that inhibits factor I release from Raji cells (3).

‡ Factor H, 40 μ g/ml.

§ $\alpha\alpha$ H, 140 μ g/ml, purified by elution from C3-agarose.

TABLE IV
Stimulation of B Cell Release of Factor I by Factor H or $\alpha\alpha$ H

B cell treatment 1 h, 37°C	Addition to B cell supernatant harvested after treatment	Factor I activity of harvested B cell supernatant as determined by Raji cell rosette assay*
		% rosettes
Buffer	Factor H‡	0
Factor H‡	Buffer	58
Factor H	Anti-factor I	0
$\alpha\alpha$ H§	Factor H	60
$\alpha\alpha$ H	Factor H and anti-factor I	0
Nonimmune IgG	Factor H	0
Fab anti-C3c	Factor H	0

* Rosette assay performed in BDVEA buffer that inhibits H receptor-mediated Raji cell release of factor I (3).

‡ Factor H concentration, 30 μ g/ml.

§ Purified anti-idiotypic eluted from goat anti-H-agarose, 300 μ g/ml.

^{||} Nonimmune IgG, 300 μ g/ml; Fab anti-C3c, 1.0 mg/ml.

free supernatant fluid was examined for I activity. The presence of small amounts of I was indicated by the anti-I-inhibitable conversion of Raji rosette-negative EC3b into Raji rosette-positive EC3bi (3). Factor I release was not detected with B cells treated with either nonimmune rabbit IgG or rabbit Fab anti-C3c. It was not possible to use the sheep IgG anti-C3b in this assay, as it agglutinated the EC3b indicator cells.

Molecular Weight Analysis of the B Cell Proteins That Bind to Either $\alpha\alpha$ H or Factor H. After intrinsic [³H]leucine labeling of Raji cell or BF cell proteins, each B cell type was solubilized with NP-40 and immunoprecipitated with purified $\alpha\alpha$ H and anti-rabbit IgG-agarose beads. Analysis of the ³H-labeled immune precipitates by SDS-PAGE revealed two proteins of 100,000 M_r and 50,000 M_r without reduction and a single protein band of 50,000 M_r with complete reduction of disulfide bonds (Fig. 2). A similar protein molecular weight profile was obtained with $\alpha\alpha$ H-immune precipitation of either Raji or BF B-cell lines, whereas no SDS-PAGE protein bands

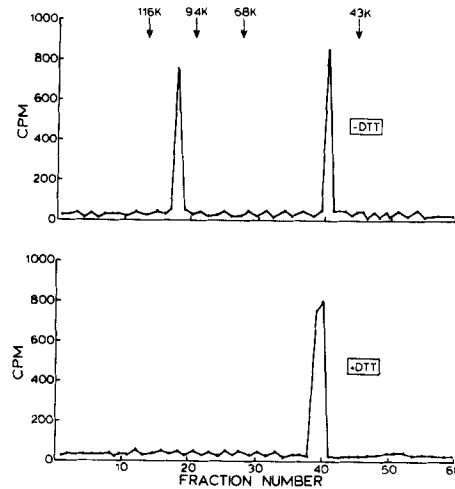


FIG. 2. $\alpha\alpha$ H immunoprecipitation and SDS-PAGE analysis of [3 H]leucine intrinsically labeled BF B-type lymphoblastoid cells. NP-40 solubilized cells were precipitated with $\alpha\alpha$ H and goat-anti-rabbit-IgG-agarose beads, and then the immune precipitates were analyzed by SDS-PAGE. The positions of known molecular weight markers run in parallel gel tracks is indicated by arrows (β -galactosidase, 116,500 M_r ; phosphorylase b, 94,000 M_r ; BSA, 68,000 M_r ; and ovalbumin, 43,000 M_r).

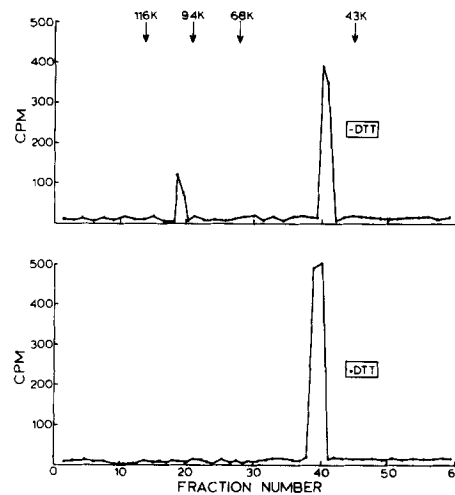


FIG. 3. SDS-PAGE analysis of [3 H]leucine intrinsically labeled Raji cell proteins absorbed and eluted from H-agarose. See legend to Fig. 2 for molecular weight markers.

were observed with either $\alpha\alpha$ H immune precipitates from [3 H]leucine intrinsically labeled HSB T cells or nonimmune IgG precipitates of labeled Raji or BF cells.

Experiments were also undertaken to characterize the B cell proteins that had factor H-binding (H receptor) activity. Spent culture media from [3 H]leucine intrinsically labeled Raji cells was chromatographed on a column of H-agarose, and then the bound proteins were eluted with 0.5% NP-40 in 0.5 M NaCl and analyzed by SDS-PAGE. As shown in Fig. 3, a similar protein molecular weight profile was obtained as with $\alpha\alpha$ H immune precipitation (Fig. 2). No comparable radioactive B

cell proteins were detected when the control eluate of a BSA-agarose column was analyzed by the same procedure. When purified C3b was electrophoresed in parallel gel tracks, its known (20) 115,000 M_r α -chain and 75,000 M_r β -chain structure was confirmed to be distinct from the B cell proteins that bound to $\alpha\alpha$ H or H.

Discussion

$\alpha\alpha$ H was shown to bind to both B lymphocyte membrane factor H receptors and C3b. The $\alpha\alpha$ H blocked the uptake of H onto B cells and triggered B cells to release factor I in the same manner as did purified H. $\alpha\alpha$ H also had H-like activity with C3b in that it served as a cofactor for I cleavage of fluid-phase C3b and potentiated I cleavage of bound C3b. SDS-PAGE analysis of B cell proteins, either immunoprecipitated with $\alpha\alpha$ H or isolated by affinity chromatography on H-agarose, revealed a similar protein molecular weight profile that probably represents the membrane H receptor.

The binding of [125 I] $\alpha\alpha$ H to both B cells and EC3b was preliminary evidence for the H receptor specificity of $\alpha\alpha$ H, as both B cells and C3b have H-binding sites. The finding that $\alpha\alpha$ H blocked [3 H]H binding to B cells and EC3b and, conversely, that H similarly blocked the binding of $\alpha\alpha$ H, indicated that $\alpha\alpha$ H bound at or near the H-binding site of B cells and C3b. Further evidence for the H-binding site specificity of $\alpha\alpha$ H was the observation that $\alpha\alpha$ H had the ability to substitute for H in reactions that are characteristic of H. Both purified H and $\alpha\alpha$ H triggered B cells to release I. In addition, $\alpha\alpha$ H not only bound to C3b, but it also functioned with I in the cleavage of C3b into iC3b. These same H-like activities could not be demonstrated with sheep IgG anti-C3b or rabbit Fab-anti-C3c. However, $\alpha\alpha$ H had only 0.1% of the activity of H in I cleavage of C3b into iC3b. This finding suggests that H function with I may depend on H interaction with I as well as with C3b and that $\alpha\alpha$ H may differ from H in that it binds only to C3b and does not also interact with I. Other studies (8) have previously suggested the existence of a trimolecular complex of C3b, H, and I that preceded iC3b formation. However, factor I also has been shown to bind to C3b-agarose (3) and EC3b (8) independently of H, suggesting that H binding to C3b may only serve to expose the I-cleavage site in C3b and that H itself may not bind to I. If this were the case, then perhaps $\alpha\alpha$ H binds to the H-binding site of C3b and either does not expose the I-cleavage site or partially covers this site.

The finding that the $\alpha\alpha$ H IgG fraction contained antibodies that were specific for C3b as well as for B cell H receptors was not unexpected. However, it was anticipated that the antibodies that were specific for the H-binding site of C3b would be distinct from those antibodies that were specific for the binding site of the H receptor. All B cell-binding activity of the $\alpha\alpha$ H IgG fraction was absorbed and subsequently eluted from C3-agarose. This finding apparently indicates that C3b and H receptors have a similar H-binding site and, thus, probably bind to the same site in the H molecule. However, if this is the case, it is not clear how complexes bearing C3b,H could trigger H receptors (3), as it would be expected that the receptor-binding site in the H molecule would be occupied by C3b. This same paradox does not exist with Clq receptors. Clq receptors have been shown to be specific for the collagen portion of Clq, whereas immunoglobulin Fc binds to the globulin portion of Clq. This allows Clq-bearing IgG or IgM aggregates to bind to Clq receptors (21). By comparison, for C3b,H complexes to trigger H receptors, the H molecule would either have to be

bivalent in identical receptor-binding sites or be able to transfer from C3b to H receptors in an activated form. No evidence has ever been presented for an activated form of factor H. However, because purified H has been noted to have a tendency to aggregate with itself (3, and S. Ruddy, personnel communication), it is possible that H interaction with bound C3b might lead to similar H aggregation, so that C3b complexes might present H dimers or larger H aggregates to cells bearing H receptors. Alternatively, even though H has been thought to be monovalent in C3b binding sites (17, 22), some of the data concerning the uptake of ^{125}I -factor H by EAC14^{oxy}23b (22) vs. EC3b (17) could be interpreted as indicating that H was bivalent. Using EAC14^{oxy}23b that contained 64,000 bound C3b molecules per cell, Conrad et al. (22) measured an uptake of only 0.5 H molecules per C3b molecule and an H to C3b affinity constant of 10^9 L/M. By contrast, Kazatchkine et al. (17), using EC3b containing 30,000 C3b per cell generated with an unstabilized convertase (C3b,Bb), observed an uptake of 1.0 H per C3b with an affinity constant of 1×10^{-7} M (10^7 L/M). C3 fixation onto EAC14^{oxy}2 is believed to generate large dense clusters of bound C3b molecules surrounding individual C4^{oxy}2 sites, because the half-life of the C4^{oxy}2 enzyme is 150 min at 32°C (23). On the other hand, because the bound C3b,Bb enzyme has a half-life of only 4 min at 30°C (24), it would be expected that only small numbers of C3b molecules would be fixed by individual unstabilized enzyme sites and that resulting C3b clusters would be small. If H were bivalent in C3b binding sites, then a single H molecule might be able to bridge two C3b molecules contained in a dense C3b cluster. This would result in an H binding of 0.5 molecules per C3b, and the cooperativity provided by dual-point attachment of H to two C3b molecules would greatly increase the H affinity constant over that observed with single-point H attachment to individual more widely spaced C3b molecules.

Because all of the various findings indicated that $\alpha\alpha\text{H}$ was probably specific for H receptors, $\alpha\alpha\text{H}$ immunoprecipitation was used in parallel with H-agarose affinity chromatography to characterize the molecular weight of B cell H receptors. Analysis by SDS-PAGE revealed two proteins of 100,000 and 50,000 M_r without reduction, and a single protein band at 50,000 M_r after complete reduction of disulfide bonds. From this data it appears possible that the membrane H receptor may consist of 50,000- M_r subunits, with the disulfide-linked subunits of the unreduced 100,000- M_r component possibly being distinct from the unreduced 50,000- M_r component. In future studies, larger quantities of H receptor will be isolated by H-agarose affinity chromatography and used for a more complete structural characterization of the receptor. Of particular interest will be determination of the carbohydrate composition of the receptor and the contribution of sugars to the binding site. A large portion of the structure of factor H is believed to be carbohydrate (1).

The main use planned for the isolated H receptor is to produce a polyclonal anti-H receptor antibody that could be converted into $\text{F}(\text{ab}')_2$ and Fab' fragments. The very small quantities of $\alpha\alpha\text{H}$ available precluded their use in generation of such pepsin-derived fragments. If both $\text{F}(\text{ab}')_2$ and Fab' anti-H receptor bound to B cell H receptors, but only the $\text{F}(\text{ab}')_2$ and not the Fab' antibody triggered I release, it would indicate that receptor cross-linkage by a bivalent H-ligand was probably required for triggering of receptor function. Such an $\text{F}(\text{ab}')_2$ anti-H receptor, as well as purified H aggregates, would also be valuable specific probes of lymphocyte H receptor-mediated functions other than I release. It is noted with considerable interest that Hammann

et al. (4) recently reported that purified human H triggers mouse spleen cell blastogenesis.

Summary

Antibody to the binding site (idiotype) of anti-factor H was shown to have specificity for both B lymphocyte membrane H receptors and C3b. Goat F(ab')₂ anti-human H was purified by absorption and elution from H agarose and used for rabbit immunization to produce anti-anti-H ($\alpha\alpha$ H). After absorption with nonimmune goat IgG, ¹²⁵I-labeled $\alpha\alpha$ H bound to B lymphocytes and to sheep erythrocytes coated with C3b (EC3b) but did not bind to T lymphocytes or to EC3d. All B cell- and C3b-specific activities of the $\alpha\alpha$ H were removed and subsequently recovered by absorption and elution of the antibody from either C3-agarose or goat-anti-H-agarose. This indicated that the $\alpha\alpha$ H probably recognized a single common antigenic structure that was shared by anti-H, C3b, and the membranes of B cells. Affinity-purified $\alpha\alpha$ H resembled H in that it bound to B cells, blocked the uptake of H onto B cell H receptors, and triggered B cells to release endogenous factor I (C3b inactivator). In addition, $\alpha\alpha$ H functioned with factor I as either a cofactor for cleavage of fluid-phase C3b or a potentiator for cleavage of bound C3b. This same spectrum of C3 binding functions could not be demonstrated with either sheep anti-C3b or rabbit-anti-C3c. Analysis by sodium dodecyl sulfate-polyacrylamide gel electrophoresis of the [³H]leucine intrinsically labeled B cell proteins reactive with the purified $\alpha\alpha$ H revealed proteins of 100,000 *M_r* and 50,000 *M_r* without reduction, and after complete reduction of disulfide bonds, a single protein band of 50,000 *M_r*. This same protein molecular weight profile was also demonstrated with labeled B cell proteins that were absorbed and eluted from H-agarose. Thus, $\alpha\alpha$ H is apparently specific for both B cell H receptors and C3b. However, because parallel analysis of C3b confirmed its known 115,000- and 75,000-*M_r* polypeptide chain structure, the H receptor is probably not C3b and shares only the structure of the H binding site with C3b.

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