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The detection and characterization of a membrane protein with Factor B-like activity on human lymphoid cells

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Summary. Factor B-like activity associated with human peripheral blood lymphocytes was first described by Halbwachs & Lachmann (1976), who employed a functional assay. In this present study, clones of B cells from patients with chronic lymphatic leukaemia (CLL) and lymphoblastoid 'Raji' cells were used. The surface of these cells was labelled with ^{125}I by the lactoperoxidase technique and the cell membrane proteins solubilized with the detergent NP40. A single polypeptide chain of molecular weight 103K was precipitated with $F(ab')_2$ anti-serum Factor B, and not with a control antibody. This 103K protein was also found if Raji cells were biosynthetically labelled with [^{14}C]-leucine. When the radiolabelled cells were incubated with cobra venom factor (CVF) and Factor D, two specific polypeptides were precipitated by $F(ab')_2$ anti-Factor B. Moreover, the larger fragment forms a complex with CVF, since it could be precipitated by anti-CVF. These events are similar to those involving serum Factor B and CVF.

INTRODUCTION

The possibility that complement components might be found associated with cell membranes was first raised by experiments on the cell-mediated lysis of chicken erythrocytes. Lymphocytes from human peripheral blood have been shown to be cytolytic *in vitro* to ^{51}Cr-labelled chicken erythrocytes (E) pretreated with IgM anti-Forssman antibody (A), and purified human complement components C1–C7, i.e. EAC1–7, but not to EAC1–3 (Müller-Eberhard, Perlmann, Perlmann & Manni, 1969; Perlmann, Perlmann, Müller-Eberhard & Manni, 1969). These results indicated that the lymphocytes might provide C8 or possibly C9, known to be necessary for cell lysis. Subsequent experiments employing $\overline{EC567}$ as target cells showed that human peripheral blood lymphocytes were cytolytic in the absence of serum C8. Moreover this reaction was inhibited by $F(ab')_2$ anti-C8 (Perlmann, Perlmann & Lachmann, 1974). It was shown more recently that in antibody-dependent cytotoxicity reactions, channels were formed in the target cell membranes by proteins associated with the membranes of the effector cells (Mayer, 1977). Moreover, such lesions were similar in electron microscopic appearance although larger in diameter, to those produced by complement (Dourmashkin, Deteix, Simone & Henkart, 1980).

Other complement components have been found to be associated with lymphocytes on functional and genetic grounds. Factor B(FB)-like activity was detected on human peripheral blood lymphocytes which, when incubated with cobra venom factor and Factor D in the absence of serum Factor B, gave rise to a C3 convertase (Halbwachs & Lachmann 1976). Anti-C4 was reported to inhibit mixed lymphocyte

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reactions (Ferrone, Pellegrino & Cooper, 1976) and so was anti-C5. Moreover C5 was reported to be synthesized when peripheral blood lymphocytes were stimulated with PHA (Sundsmo & Müller-Eberhard, 1979).

Studies on complement genetics have shown that serum C2, C4 and FB polymorphisms are closely linked to the HLA-B locus (Hobart & Lachmann, 1976; Alper 1976; Teisberg, Olaisen, Jonassen, Gedde-Dahl & Thorsby, 1977; Allen 1974). Since products of the major histocompatibility system have been shown to be involved in cellular immune responses, it has been speculated that there may be a role for complement in cellular interactions too.

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This paper describes the results of experiments designed to answer the following questions: (1) is the Factor B-like activity produced by a membrane protein rather than adsorbed serum Factor B? (2) Is the membrane protein synthesized by lymphoid cells? (3) Is this protein an integral membrane protein, as opposed to a protein that is being secreted? (4) Are there other complement components detectable on the lymphoid cell membrane?

MATERIALS AND METHODS

Chemicals

All chemicals used, apart from those specified separately were Analar grade from British Drug Houses.

Cell culture

Mycoplasma-free Raji cells were cultured in large numbers in RPMI 1640 (Gibco) contained in Winchester bottles placed on rotators at 37°. Ten percent foetal calf serum previously heated at 56° for 30 min, 15 mM HEPES, penicillin (1000 u./ml), and streptomycin (1000 μg/ml) were added to the culture medium.

Separation of lymphocytes from the peripheral blood of chronic lymphocytic leukaemia patients

Patients suffering from chronic lymphocytic leukaemia (CLL) provided large numbers of monoclonal B cells. Freshly drawn blood was layered over an equal volume of Ficoll-Paque (Pharmacia) and centrifuged at 1500 *g* for 25 min. The lymphocytes were harvested from the interfacial band.

Antisera

Antisera to complement components were raised in rabbits except anti-FB, which was raised in a sheep. All antisera used were monospecific on double diffusion and the IgG fractions (prepared by DEAE chromatography) were used. Specific antibody was purified as $F(ab')_2$ as described by Lachmann (1971). The IgG fractions were absorbed with human red cells (0+ ve), followed by CLL or Raji cells before use.

Factor D (FD) was purified by Sephadex G100 chromatography as described by Lachmann & Hobart (1978). Cobra venom factor (CVF) was purified as described by Ballow & Cochrane (1969).

Purification of serum Factor B

This procedure involved three fractionations and was modified from that described by Lachmann & Hobart (1978).

(a) Freshly drawn blood from a donor was allowed to clot in a glass container. The 40%–70% ammonium sulphate precipitated fraction from the serum was taken for further purification, which was all performed at 4°. This fraction of serum was concentrated and fractionated on methyl cellulose CM32 (Whatman). The buffer used was 10 mM phosphate, 10 mM EDTA and 5 mM sodium azide at pH 6, conductivity 2·8 mmhos/cm^2 at 23°. A linear gradient of 0·3 M sodium chloride in the running buffer was applied over five column volumes and FB activity in the fractions was detected by the haemolytic assay described in Lachmann & Hobart (1978). FB elutes from 10·5 mmhos to 13 mmhos/cm^2 at 23°. There was an overlap of C2 activity which was also detected on a haemolytic C2 plate (Lachmann & Hobart 1978). Since C2 elutes from 9·5 mmhos to 10·5 mmhos, two pools were made; one containing FB activity only and one with C2 and FB activity. Sodium dodecyl sulphate (SDS)-polyacrylamide gel analysis showed that the main contaminant of these pools was IgG.

(b) The fractions pooled from the CM column were dialysed and fractionated on DE32 in 20 mM phosphate pH 7·2, containing 5 mM azide. The conductivity at 23° was 2·6 mmhos/cm^2. A linear gradient of 0·3 M sodium chloride in the eluting buffer was used over five

column volumes. C2 activity was found separately from FB activity, the latter eluting from 3·6 mmhos to 5·6 mmhos. SDS gel analysis still showed contaminants in the pooled FB fraction.

(c) The final step consisted of fractionation on a hydroxylapatite column manufactured essentially as described by Main, Wilkins & Cole (1959). The running buffer consisted of 10 mM phosphate, 5 mM disodium EDTA and 1 mM azide at pH 6 with a conductivity of 1·7 mmhos/cm^2 at 23°. A linear gradient of 0·3 M phosphate was applied over five column volumes and FB was pooled by activity as before. Five to ten percent SDS-polyacrylamide gel analysis showed one single band of apparent mol. wt 103 K on 7·5% gel and 12% gel (K = 1000 Daltons). The overall yield was about 6%.

Surface iodination of cell membrane proteins using lactoperoxidase

The method used in these experiments was modified from that described by Haustein, Marchalonis & Harris (1975) in order to increase the percentage uptake of ^{125}I by the cells. Aliquots of 100% viable cells (measured by their ability to degrade fluorescein diacetate) were resuspended in 200 μl PBS 10^{-5}M KI. carrier free ^{125}I (4 mCi) were added to each aliquot, followed by 20 μl 0·03% H_2O_2 and 40 μl of lactoperoxidase (5 mg/ml, Sigma). After incubation at 30° for 3 min, more H_2O_2 and lactoperoxidase were added. Iodination was terminated by dilution with 10^{-3} M KI/PBS/Az. The iodinated cells were washed four times and kept at 4° throughout.

Extraction of membrane proteins for analysis

Two methods were employed:

(1) Membrane solubilization and analysis. Freshly iodinated cells (10^8) were solubilized in 0·5% NP40 containing 10 mM azide, 10 mM DFP, 0·1 mg/ml Trasylol (5·4 Kallikrein u./mg), 10 mM EDTA and 1 mM iodoacetamide in a final volume of 0·7 ml. After 30 min at 4° the mixture was centrifuged at 700 *g* for 10 min to remove intact nuclei, and the supernate recentrifuged in a microfuge to remove microsomes and endoplasmic reticulum. The supernate was taken immediately for indirect specific immune precipitation, employing F(ab′)$_2$ sheep anti-FB and rabbit anti-sheep IgG. As a control for non-specific precipitation of radioactive material, sheep F(ab′)$_2$ anti-T4 phage and rabbit anti-sheep IgG were used. The precipitates were washed in 0·2% SDS/PBS/Az and analysed on 7·5%–10% SDS-PAGE after Laemmli (1970), using vertical slab gels. After electrophoresis, the protein bands in the gel were fixed in 25% TCA for 30 min and stained with Coomassie brilliant blue in methanol:acetic:water (20:5:75). The gel was dried onto filter paper and subject to enhanced autoradiography as described by Laskey & Mills (1977).

(2) Extraction of membrane factor B (membrane FB) by cobra venom. Aliquots of 10^8 freshly iodinated cells were resuspended in 36 μl Glasgow Eagle's/-HEPES medium, and divided into two groups: test and control. To the test tubes were added 20 μl FD (diluted 1:4) and 20 μl CVF, whereas 10 μl of 0·2 M EDTA pH 7·2 was added to control tubes before the addition of FD and CVF. The cells were incubated at 37° for 20 min with mixing and removed by centrifugation. The supernates were then taken for indirect double precipitation using (a) sheep F(ab′)$_2$ anti-FB and rabbit anti-sheep IgG and (b) rabbit anti-CVF followed by goat anti-rabbit IgG. The precipitates were washed and analysed as described above in 1.

Incorporation of [^{14}C]-leucine into membrane proteins and their analysis

The method used was essentially that described by Ballou, McKean, Freedlender & Smithies (1976). Raji cells were depleted of leucine for 48 hr and then resuspended in medium containing [^{14}C]-leucine (1 μCi/ml) for 18 hr at a concentration of 5×10^6 cells/ml. The viability of the cells after labelling was 95%. The spent medium was concentrated ten to twenty times using a minicon B15 filter (Amicon) and tested for complement components on double diffusion agarose plates, using antisera raised to serum components, and in the presence of 10% normal human serum as carrier. Precipitation lines were detected by autoradiography of these dried agarose plates.

The labelled cells were then subject to either membrane solubilization and analysis, or to reaction with CVF, as described above. The dried acrylamide gels were subject to fluorography (Laskey & Mills, 1975) for the identification of membrane proteins specifically precipitated by F(ab′)$_2$ anti-FB. The proteins from the gels were also eluted using 0·5 ml NCS/water (9:1) for each 2 mm strip (Basch, 1968). Two millilitres of scintillation fluid (PPO/toluene) were added to each eluate and the radioactivity measured.

RESULTS

The extraction of membrane FB by CVF from ^{125}I-labelled cells and its characterization

Freshly iodinated CLL and Raji cells were incubated with CVF and FD. The supernates were then taken for double immune precipitation and the precipitates analysed as described in Methods. Figure 1 shows the enhanced autoradiographs of the acrylamide gels analysing the F(ab')$_2$ anti-FB and anti-CVF precipitates formed in cell supernates with and without EDTA present. In the case of CLL cells, the track containing the F(ab')$_2$ anti-FB precipitate formed in the absence of EDTA revealed two ^{125}I-bands of mol. wt 89K and 33K, which are not seen in the track containing anti-FB precipitate formed in the presence of EDTA. In the case of Raji cells, the anti-FB precipitate from the test supernate contained a specific

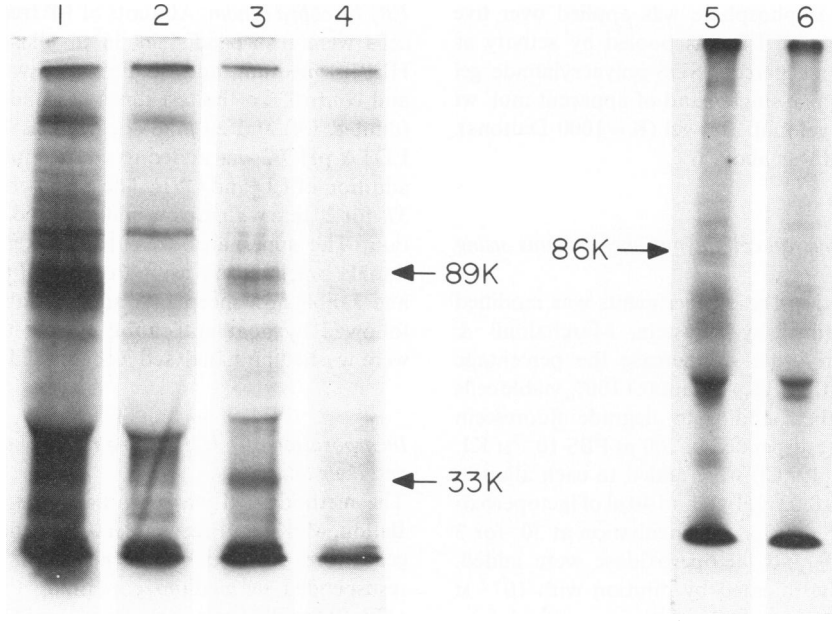

Figure 1. Autoradiographs of anti-FB and anti-CVF precipitates from membrane solutes. The Table below shows the reagents used in each track, and the molecular weights of specific bands.

	CLL				RAJI	
Track	1	2	3	4	5	6
Reagents						
Cells	+	+	+	+	+	+
CVF	+	+	+	+	+	+
D	+	+	+	+	+	+
EDTA	−	+	−	+	−	+
Anti-FB	−	−	+	+	+	+
Anti-CVF	+	+	−	−	−	−
MW of specific bands:						
Raji	−	−	−	−	86K	−
CLL	89K	−	89K	−	−	−
	−	−	33K	−	−	−

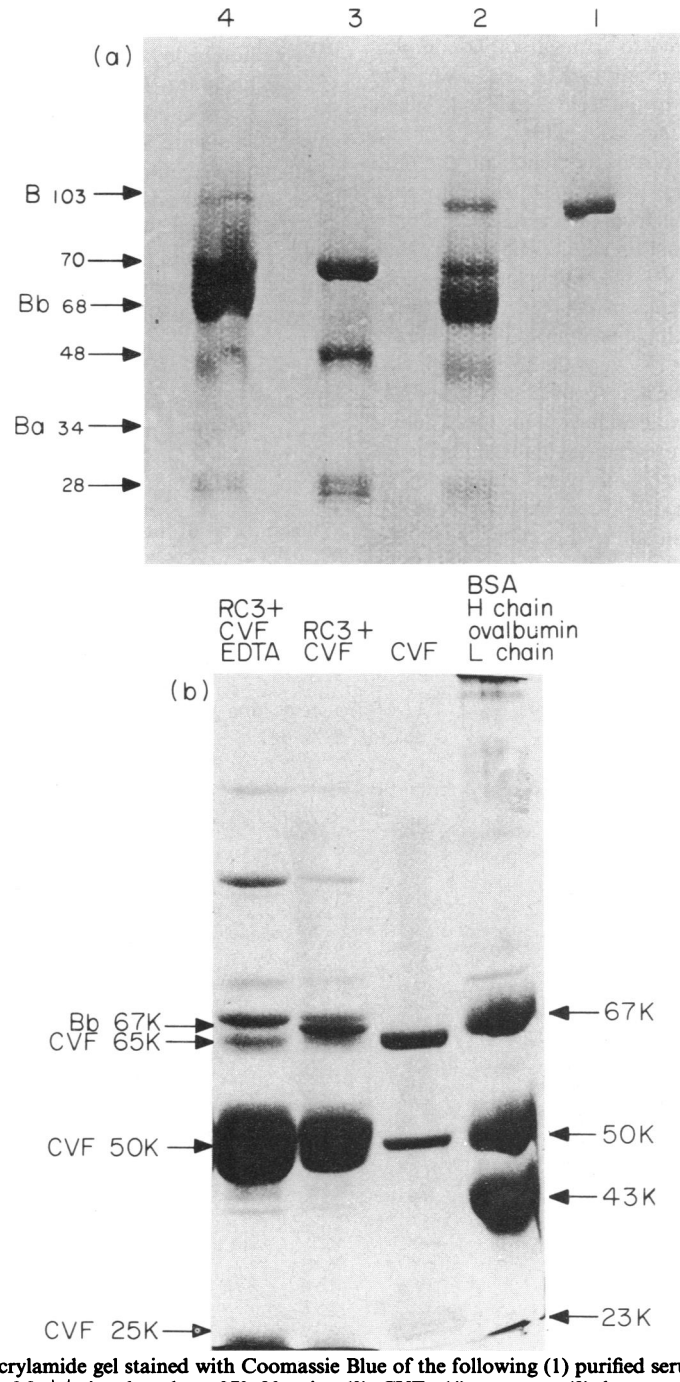

Figure 2. (a) SDS-polyacrylamide gel stained with Coomassie Blue of the following (1) purified serum FB, (2) purified serum FB+CVF+D+10 mM Mg^{++} incubated at 37° 20 min, (3) CVF, (4) same as (2) but overloaded to show Ba. (b) SDS-polyacrylamide gel of anti-CVF precipitates. RC3 = normal human serum depleted of C3 by precipitation with $F(ab')_2$ anti-C3 in the presence of 10 mM EDTA, and subsequently reconstituted with 20 mM Mg^{++}. Bb (mol. wt 66K) was precipitated when RC3 was incubated with CVF in the absence of EDTA.

band of mol. wt 86K. When anti-CVF was used, the larger fragment appeared to form a stable complex with CVF since it was precipitated by anti-CVF. The results are summarized in the legend to Fig 1. When purified serum FB was incubated with CVF, FD and Mg^{++} it was cleaved into two fragments: mol. wt 68K and 34K. Furthermore this larger fragment was precipitable by anti-CVF from serum which has been depleted of C3 before reaction with CVF (Fig. 2). Thus the reaction between CVF and cells produced analogous products to those from the reaction between CVF and Serum FB, with the exception of an apparent difference of 20K in the mol. wt of the larger fragment.

As a further control for non-specific precipitation of radioactive material, ovalbumin and anti-ovalbumin were employed to form precipitates in test and control supernates. No specific bands were found. When human red cells, known to possess no functional FB activity were used instead of Raji/CLL cells, no specific ^{125}I bands were detected using the same experimental procedures.

Extraction of membrane FB by detergent (NP40)

^{125}I radiolabelled CLL or Raji cells were solubilized The $F(ab')_2$ anti-FB and the control, $F(ab')_2$ anti-T4 phage, indirect double immune precipitates formed in the lysates were analysed by 7·5%–10% SDS-PAGE and enhanced autoradiography as described in Materials and Methods above. A single faint radioactive band of mol. wt 103K was found repeatedly only in the tracks containing anti-FB precipitates (Fig. 3). This mol. wt estimation on 7·5% gel was similar to that of purified serum FB (Fig 2a).

An order of magnitude estimate of the number of molecules of membrane FB per CLL and Raji cell was obtained as shown in Table 1. This shows that there are only 10^2–10^3 molecules of membrane FB per CLL or Raji cell.

Biosynthesis of membrane FB

Raji cells were depleted of leucine for 48 hr and then incubated with [^{14}C]-leu for 18 hr as described in

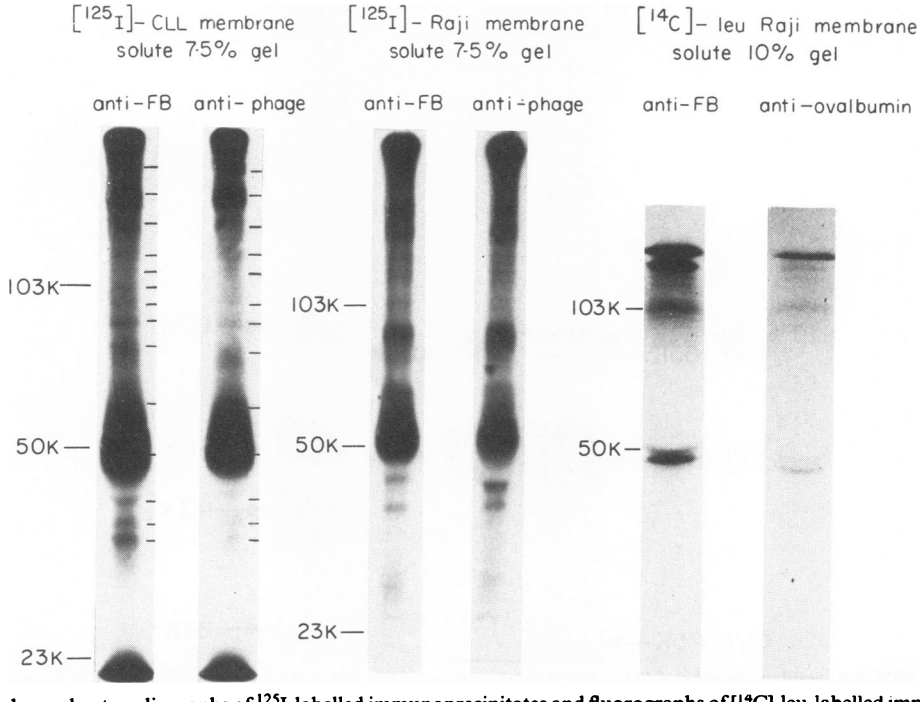

Figure 3. Enhanced autoradiographs of ^{125}I-labelled immunoprecipitates and fluorographs of [^{14}C]-leu-labelled immunoprecipitates. The antibody used is shown at the head of each track. The autoradiographs are overexposed to show the faintest bands, and so there is a high noise:signal ratio. The loss in definition of the bands is consistent with the use of enhanced autoradiography. A line drawing is added at the side of each track to aid comparison.

Table 1. Estimation of the number of FB molecules per cell

	Radioactivity under FB peak/ area under whole track	Radioactivity of the gel track in c.p.s. (from 10^8 cells)	Radioactivity of FB peak in c.p.s. (from 10^8 cells)	Labelling ratio atoms of I/10^5 mol. wt protein	Number of FB molecules per cell
CLL	0·017	213	3·6	1/124	86
	0·032	797	25·5	1/124	489
	0·010	1255	12	1/124	285
	0·025	1533	38·5	1/124	116
Raji	0·04	91	3·6	1/291	201
	0·015	2007	30	1/291	1674
	0·02	153	26	1/291	145
	0·03	132	3·7	1/291	201

A densitometry profile on graph paper was obtained for each track on the autoradiographs. The paper under the curve was cut out and weighed. The area under the FB peak was then cut out and weighed, thereby giving an estimate of the ratio of the radioactivity of the FB band compared to the whole track. The labelling ratio was obtained as described in Table 2.

Since the number of iodine atoms producing one disintegration per second is $1{\cdot}037 \times 10^7$, the radioactivity of the FB peak can be expressed in terms of numbers of ^{125}I atoms, and then the number of membrane FB molecules per cell can be estimated: no. of FB molecules per cell = FB counts (c.p.s.) $\times$ (37/20) $\times$ $1{\cdot}037 \times 10^7 \times (1/10^8)$ $\times$ labelling ratio.

Materials and Methods. The viability of the cells after 18 hr was 95%. The proteins from these labelled cells were solubilized with NP40. Double immune precipitates were formed in the lysates using sheep F(ab′)$_2$ anti-FB and rabbit anti-sheep IgG. As a control for non-specific precipitation of radioactive material, ovalbumin was added followed by anti-ovalbumin at optimal proportions, which was previously obtained from double diffusion on agarose plates. A radioactive band of mol. wt 103K was seen in the F(ab′)$_2$ anti-FB precipitate and not in the ovalbumin precipitate (see Fig 3). There were two other bands which appear in the test column and not in the control but these may be contaminants in view of the results obtained with incubating [^{14}C]-leu cells with CVF + D.

When Raji cells were labelled with [^{14}C]-leu, incu-

Table 2. The estimation of the number of membrane FB molecules per Raji cell labelled with ^{14}C leucine

1. Specific activity of L-(U-^{14}C) leucine (Radiochemicals, Amersham)	354 Ci/mole
2. Counts per second per 1 μCi of [^{14}C]-leu	$2{\cdot}3 \times 10^4$
3. Total radioactivity of anti-FB precipitate (digested by NCS)	32 c.p.s.
4. Radioactivity of FB bands	1·28 c.p.s.
5. No. of moles of [^{14}C]-L-leucine in FB bands (calculated from 1, 2 and 4)	1·6 c 10^{-4} nmoles
6. Total membrane protein per 10^8 Raji cells (measured)	2·6 mg
7. Radioactivity of 10^8 cells (measured)	$3{\cdot}5 \times 10^5$ c.p.s.
8. Radioactivity per mg protein (7/6)	$1{\cdot}3 \times 10^5$ c.p.s.
9. No. of moles of [^{14}C]-L-leucine per mg protein	16 nmoles
10. Assuming uniform uptake of leucine/mass of protein, no. of moles of [^{14}C]-L-leucine/mole of protein with MW of 10^5	1·6
11. No. of molecules of FB on 10^8 cells	(5)/(10) $= 6 \times 10^{10}$
12. No. of FB molecules per cell	600

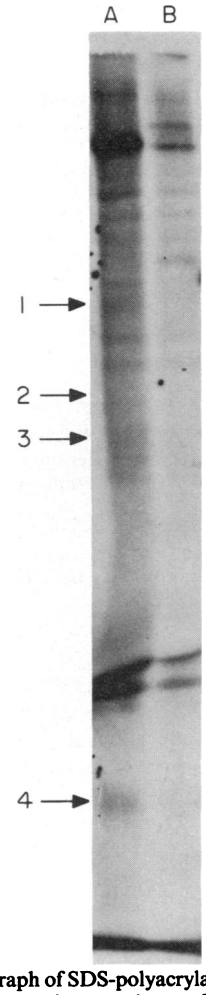

Figure 4. Fluorograph of SDS-polyacrylamide gel of anti-FB precipitates from membrane solutes of cells labelled with [^{14}C]-leucine. In Track A, cells+CVF+D. In Track B, cells+EDTA+CVF+D (i.e. control). 1=103K; 2=84K; 3=72K; 4=31K.

bated with CVF+FD and treated in the same manner as described in Materials and Methods the following results were obtained. Radioactive bands of mol. wt 103K, 84K, 72K and 31K were seen in the F(ab′)$_2$ anti-FB precipitate formed in the supernate without EDTA and not in the supernate containing EDTA (Fig. 4). The reaction of ^{14}C-labelled Raji cells with CVF is therefore analogous to that of ^{125}I-labelled Raji cells, and ^{125}I-labelled CLL cells. The band of 103K was present probably due to incomplete cleavage by Factor D after membrane FB was extracted by CVF. The 84K band was very faint and diffuse, and was probably derived from the same fragment as the 72K band. These apparent molecular weight differences will be discussed. The 31K fragment corresponds to the 33K fragment seen in the CVF extracts from ^{125}I-labelled CLL cells (Fig. 1) and to the serum Ba fragment (Fig. 2a).

In order to estimate the number of membrane FB molecules per cell, the dried gel columns were cut into 2 mm strips after the specific FB bands were cut out, and then digested with NCS as described. Table 2 shows the estimation of the number of FB molecules per Raji cell. There are 6×10^2 molecules of FB molecules per Raji cell and this value is within the range 10^2–10^3 obtained previously using ^{125}I-labelled cells (see Table 1).

The spent radioactive culture media were concentrated ten to twenty times on a Minicon B15 filter (Amicon) after the 18 hr incubation period. These concentrates were tested on double diffusion plates in agarose (after Ouchterlony) for complement components, using antisera against FB, FD, C2, C3, C4, C5, C6, C7, C8, I and H. Ten percentage normal human serum was added as carrier to aid immunoprecipitation. Autoradiographs of these plates did not show any radioactive lines whilst media from some haemic lines (unpublished results) showed radioactive precipitation lines with C3 and C5. These results suggest that complement components were not secreted by Raji cells in the 18 hr incubation period.

Other cell surface complement components

Antisera raised to serum C2, C3, C4, C5, C6, C7, C8 serum I, D and H were used to detect any cross-reacting ^{125}I-labelled membrane antigens, employing the same procedures as those used for the detection of membrane FB. Surface labelled Raji and CLL cells were solubilized and indirect immune precipitation performed using rabbit anti-complement IgG and goat anti- rabbit IgG. The volume of anti-complement IgG used in each case was capable of precipitating at optimal proportions between 1–54 μg of serum complement components. As a control for non-specific precipitation of radioactive material, ovalbumin followed by anti-ovalbumin were added and the precipitate was analysed alongside those using specific antisera. F(ab′)$_2$ anti-FB was also used in each experiment as a positive control. No specific radioactive bands were precipitated from [^{125}I]-cell lysates.

DISCUSSION

The functional FB-like activity associated with lymphoid cells described by Halbwachs & Lachmann (1976) has been shown to be due to a protein on these cell membranes, i.e. membrane FB. It is not adsorbed serum FB because it has been detected on Raji cells cultured in the absence of serum FB. Moreover it is synthesized by Raji cells *in vitro*.

Membrane FB is probably an integral membrane protein. It is not secreted and it is not eluted by EDTA or hypotonic solution (0·01 M Tris HCl pH 7·2). In addition, the fact that it is solubilized by a detergent may be indirect evidence that it is amphilic. It was not possible to show whether membrane FB was amphilic by charge shift crossed immunoelectrophoresis (Helenius & Simons 1977; Bhakdi, Bhakdi-Lehnen & Bjerrum, 1977) since there was so little membrane FB per cell. The order of magnitude estimations of the number of molecules of membrane FB per cell showed that there were approximately 10^2–10^3 molecules per Raji or CLL cell. This estimate is based on three assumptions: there was non-selective radiolabelling, all membrane proteins have a mol. wt of 10^5, and the calculations did not take into account normal membrane activities like pinocytosis and the shedding of antigens. Thus there appeared to be one to two orders of magnutude less membrane FB expressed on the cell membrane than there is Ig, since the number of membrane immunoglobulins has been quoted in the range of approximately 10^4–5×10^5 per CLL cell (Loor 1977). It is noteworthy that the immunoprecipitation and autoradiographic techniques employed for the characterization of cell surface IgM has been adequate for IgM. However, when applied to antigens which are present in smaller numbers such as membrane FB, these techniques are limited by the high noise to signal ratio in the enhanced autoradiographs which are necessary in order to visualize the faint bands. Table 3 summarizes the various molecular weight determinations of serum, membrane FB and their fragments.

Both serum FB and detergent solubilized membrane FB were cleaved on incubation with CVF and FD, and the larger fragments formed complexes with CVF, precipitable by anti-CVF. However, the larger membrane fragment was apparently 20K larger than the serum Bb, whether it was isolated from ^{125}I-labelled cells or ^{14}C-labelled cells. Therefore, it is likely that membrane FB is larger and cleaves into two peptides like serum FB but the larger fragment contains an extra membrane 'tail', analogous to the IgM membrane tail in cell-bound IgM, described by Alt, Bothwell, Knapp, Siden, Mother, Koshland & Baltimore (1980), Rogers, Early, Carter, Calane, Bond, Hood & Wall (1980). This was not apparent on molecular weight determination of NP40 solubilized membrane FB, but this discrepancy may be due to alteration in the lipid composition of the membrane protein by NP40. Alternatively, the larger fragment could have the same amino acid composition as serum Bb, but have more carbohydrate attached, thus retarding its mobility on SDS-polyacrylamide gels and giving it a higher apparent molecular weight.

An alternative explanation for the existence of membrane FB is that it may be a product of a duplication of the gene coding for serum Factor B. One duplication of this gene already exists since C2 and FB are most probably products of duplicated

Table 3. The various molecular weight determinations of serum FB, membrane FB and their fragments

	Molecular weights of bands from specific $F(ab')_2$ anti-FB precipitation	Molecular weights of bands from anti-CVF precipitation
Purified serum FB	103K	ND
Purified serum FB+CVF+D	2 bands: 68K, 34K	1 band 68K
Membrane FB from cells solubilized by NP40		
(1) ^{125}I-labelled	103K	ND
(2) ^{14}C-labelled	103K	ND
Membrane FB+CVF+D		
(1) ^{125}I-labelled	2 bands: 89K & 33K	1 band: 89K
(2) ^{14}C-labelled	4 bands: 103K, 84K, 72K, 31K	ND

ND, not done.

genes in view of their physicochemical properties, reaction patterns, HLA linkage, and limited sequence homology (Kerr, 1979). It would be interesting if allotypes of membrane FB could be found by using sensitive isoelectricfocusing technique and perhaps incorporating a zwitterionic detergent like Empigen BB in the gel to keep the proteins soluble.

It was not possible to demonstrate other membrane-associated complement components using the same techniques. The antibodies used were raised to serum components and perhaps only antiserum FB cross-reacts with membrane FB. The double precipitation method produces a high noise to signal ratio on enhanced autoradiographs and perhaps other components are present in smaller quantities than FB but remain undetected because of the high background in the autoradiographs. It would be interesting to see if monoclonal anti-complement antibodies coupled to protein A Sepharose would uncover fresh components on the membrane.

It is noteworthy that Wilson & Coombs (personal communication) could not detect any FB on the surface of human peripheral blood lymphocytes, obtained from blood taken into EDTA or heparin. They devised a sensitive direct rosette technique to detect cell surface antigen using the same anti-FB as employed in the experiments described (Wilson, Karoki & Coombs, 1978). These results corroborated with those obtained by Halbwachs & Lachmann (1976): preincubation with anti-FB did not block cell FB-like activity. Therefore the antigenic site of membrane FB is likely to be hidden and appears to be distinct from the CVF binding site. Wilson & Coombs could not detect any complement component associated with cell surfaces apart from C4, which was found to be adsorbed from serum. Similarly, although Ferrone, Pellegrino & Cooper (1976) described C4 associated with lymphoblastoid cells, it is increasingly clear that C4 is adsorbed onto the lymphocytes in view of the work by Tilley, Romans & Crookston (1978) on the blood group antigens Chido and Rodgers, which are isotypes of C4. They showed that Chido-negative red cells became Chido positive in the presence of positive sera. It is likely, therefore, that anti-complement sera raised to serum components do not cross-react with membrane antigens on the cells examined apart from anti-FB, in contrast to the detection of C5 by Sundsmo & Müller-Eberhard (1979) mentioned above in the Introduction.

The function of membrane FB is unclear. It has been suggested that membrane FB is the C3b receptor but it is unlikely to be so for the following reasons. Firstly, classical C3b receptor activity is lost on trypsin treatment of the cells but FB activity remains. Second, many Burkitt's lymphoma lines have FB activity but lack C3b receptors. Third, anti-FB does not inhibit the binding of C3b by the C3b receptor. Fourth, human erythrocytes have receptors for C3b but lack FB activity. Fifth, there are murine strain differences in the levels of FB positive cells but none in the level of C3b receptor cells, and finally it was found that the C3b receptor cannot be blocked by CVF (McConnell & Lachmann 1976). At present one can only speculate on the role of membrane FB. It is still possible that FB has a subtle link with cell bound C3b. The small quantity of FB detected need not necessarily mean that it is insignificant, since its turnover rate is unknown.

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