Clin. exp. Immunol. (1980) 39, 477–485.

Human lymphocyte markers defined by antibodies derived from somatic cell hybrids

I. A HYBRIDOMA SECRETING ANTIBODY AGAINST A MARKER SPECIFIC FOR HUMAN B LYMPHOCYTES

D. A. BROOKS, I. BECKMAN, J. BRADLEY, P. J. MCNAMARA, MIRIAM E. THOMAS & H. ZOLA *Department of Clinical Immunology, Flinders Medical Centre and Flinders University of South Australia*

(*Accepted for publication* 2 *July* 1979)

SUMMARY

A hybridoma has been isolated from the products of fusion of a myeloma cell line with spleen cells from mice immunized with a human B cell line. After cloning, the hybridoma secretes antibody with the following properties: (i) Human B-lymphoblastoid cell lines react with the antibody while T and null cell lines do not. (ii) The antibody reacts with the majority of leucocytes in the blood of patients with CLL, but with a minority of cells in the blood of patients with AML or ALL of the null or T type. (iii) The antibody reacts with 9–21% of mononuclear cells in normal peripheral blood. The reacting cells are not T cells and overlap extensively with cells identified as B cells by other markers.

The antigen identified by this antibody appears to be distinct from known B cell markers, and is put forward as a new B cell marker with diagnostic potential.

INTRODUCTION

The specialization of function amongst lymphocytes has been well demonstrated in experimental animals, and it is clear from 'experiments of nature' that division of labour amongst lymphocyte subpopulations occurs also in man (reviewed by Cooper *et al.*, 1973). Mouse lymphocytes carry a number of alloantigens, which behave as differentiation antigens in that they characterize the state of differentiation of the cells. In the mouse it appears that different functional subsets of lymphocytes express distinct sets of these differentiation antigens.

This information derives largely from cross-immunization experiments between inbred strains of mice and from adoptive transfer experiments, techniques which are not applicable to humans. In man, functional experiments must be done largely *in vitro*, and the study of differentiation antigens has been difficult because, in cross-species immunization, antigens common to all human lymphocytes dominate the response and antisera are not specific. Thus, for example, the preparation of an antiserum specific for human T lymphocytes has been difficult to achieve. Antisera have to be absorbed extensively with B lymphocytes and the absorbed antisera have rather weak reactivity and only relative specificity (see for instance Zola, 1977a). Certain manoeuvres, such as coating the immunizing cells with antibody against common determinants (Brown, Capellaro & Greaves, 1975; Zola, 1977b), the induction of partial tolerance to the common antigens (Zola, 1977a), or partial purification of the antigens (Goodfellow *et al.*, 1976) help to varying degrees. The preparation of antisera with a higher discriminatory power, capable of reacting for instance with a functional subset of lymphocytes, would appear to be extremely difficult by methods of this type.

Correspondence: Dr H. Zola, Department of Clinical Immunology, Flinders Medical Centre, Bedford Park, South Australia 5062.

0099-9104/80/0200-0477 $02.00 © 1980 Blackwell Scientific Publications

The development by Kohler & Milstein (1975) of the hybridoma method for preparing monoclonal antibodies offers one possible solution to this problem. Although the serum of an animal immunized with human lymphocytes contains a mixture of antibodies, amongst which the antibodies against common antigens predominate, each individual plasma cell can be expected to produce a single type of antibody. The majority of spleen cells will be making antibody against the common determinants, but by combining the fusion technique to produce hybridoma colonies with rapid screening techniques to assess specificity, it is possible to select hybridoma colonies producing antibody against specific determinants (Levey, Dilley & Lampson, 1978; Hammerling *et al.*, 1978).

This paper describes the preparation and properties of a hybridoma antibody reacting with human B lymphocytes. The spectrum of reactivity of this antiserum is examined and the expression of the antigen is compared with the expression of the known B lymphocyte markers.

MATERIALS AND METHODS

Media. RPMI medium: RPMI 1640 supplemented with 10% foetal calf serum, glutamine (2 mM), penicillin (100 iu/ml) and streptomycin (100 iu/ml) (Flow Laboratories).

HAT medium: RPMI medium containing hypoxanthine (136 μg/ml), aminopterin (0·19 μg/ml) and thymidine (3·88 μg/ml).

HT medium: As HAT medium but aminopterin omitted.

PBS: Dulbecco's phosphate-buffered saline, free of Ca^{++} and Mg^{++}.

Cell lines. The mouse myeloma line used for hybridization was the BALB/c line P3-NS1-AG4-1 generally known as NS1 and originally derived by Kohler & Milstein (1975).

The human lymphoblastoid cell lines BALM 1, BALM 2, NALM 6, RPMI 8866, RPMI 8392, RPMI 1788, HRIK, CCRF HSB2, CCRF CEM, JM, REH and KM3 were kindly provided by Dr J. Minowada of the Roswell Park Memorial Institute, Buffalo, USA. The human myeloma line U226B was kindly supplied by Dr C. M. Steel, Edinburgh, UK. All the cell lines were maintained in RPMI medium, at 37°C in a humidified atmosphere containing 5% CO_2 in air.

Human lymphocytes. Peripheral blood lymphocytes were obtained by fractionating heparinized blood on Ficoll-Hypaque (Böyum, 1968).

Peripheral blood lymphocytes were fractionated into T and non-T fractions using a scaled-up modification of the nylon wool column method of Trizio & Cudcowicz (1974).

Immunization. Male BALB/c mice obtained from the Institute of Medical and Veterinary Sciences, Adelaide, aged 6 to 8 weeks at the start of the experiment, were immunized by an i.p. injection of 2×10^7 antibody-coated BALM/1 cells in 1 ml PBS. The coating antibody was a mixture of hybridoma culture supernates which reacted non-specifically with human lymphocytes. Coating was carried out as described previously (Zola, 1977b). Four weeks after immunization a second injection of coated cells was given and four days later spleens were removed for hybridization.

Hybridization. Hybridization was carried out essentially as described by Galfre *et al.* (1977). Spleens from immunized mice were gently teased apart in RPMI medium to prepare a single-cell suspension. Erythrocytes were lysed by centrifuging the cells (200 *g*, 5 min) and resuspending them in Gey's haemolytic medium (5 ml per spleen) for 5 min at room temperature. The cells were centrifuged again and washed once in RPMI medium. Myeloma cells, which had been diluted to a concentration of 3×10^5 per ml the previous day to ensure active growth, were mixed with spleen cells in a ratio of 10 spleens cells per myeloma cell. The mixed suspension was centrifuged and supernatant removed. The cell pellet was resuspended in 1 ml of a sterile solution prepared by dissolving 10 g polyethylene glycol (British Drug Houses; 4000 molecular weight) in 14 ml of PBS containing 15% dimethyl sulphoxide. After resuspension, the cells were allowed to stand for 1 min and then diluted by drop-wise addition of 3 ml RPMI medium over the next 8 min; a further 10 ml was added over a further 8 min and the cells were then centrifuged and resuspended in RPMI medium. Cells were incubated at 37°C in 5% CO_2, for 4 hr, centrifuged, resuspended at a concentration of 2×10^5 myeloma cells per ml in prewarmed HAT medium, washed once more and 1 ml aliquots dispensed into wells of tissue culture cluster plates (Costar 3524). Cultures were kept at 37°C in a humidified atmosphere containing 5% CO_2 and examined using an inverted microscope for the growth of colonies. Colonies could be observed microscopically after 5 to 6 days and macroscopically after 8 days. Cultures were fed by gentle addition of 1 ml of HAT medium 4 days after hybridization and were fed again by removing 1 ml of medium and replacing with fresh HT medium 10 days after hybridization. Thereafter, feeding was carried out when judged necessary by the colour of the medium and concentration of cells. When the medium showed considerable yellowing, supernatant samples were taken for testing against the immunizing cell line. Positive colonies were scaled up for further testing and cloning.

Cloning. Hybrids were cloned by a limiting dilutions technique in liquid medium in the presence of normal spleen cells acting as a feeder layer. Spleen cells from non-immunized mice were prepared, as described for hybridization, at a concentration of 1–2 million viable cells per ml in HT medium. Hybrid cells were added at a nominal concentration of 1 or 2 cells per ml and 1 ml aliquots of the mixture were plated out into culture wells. The plates were incubated at 37°C in 5% CO_2 for 3 days and then fed with 1 ml of HT medium. Microscopic colonies generally appeared 7 days after setting up the cloning and by 10 days macroscopic colonies were visible.

Out of twenty-four wells set up, sixteen showed no growth, five had single colonies, and three had multiple colonies. To ensure monoclonality, only the wells with single colonies were selected for expansion and testing.

Test pool. A pool of supernatant was prepared and stored frozen in small aliquots. All subsequent tests were done on this pool. The mouse immunoglobulin concentration of this pool was estimated to be 20 μg/ml, by immune precipitation analysis using a laser nephelometer assay (kindly performed by Mr R. Gale).

Fluorescent antibody test. Indirect immunofluorescence was carried out in PBS containing 0·02 M sodium azide, as described previously (Zola, 1977a). Briefly, cells (50 μl; 10^7/ml) were incubated on ice with antibody (50 μl) for 20 min, washed twice in cold PBS/azide, resuspended in 50 μl conjugated anti-globulin, incubated on ice for a further 20 min, washed twice as before and finally resuspended in 50% PBS:50% glycerol and applied to microscope slides. The fluorescent anti-immunoglobulins used were either rabbit anti-rat (cross-reacting with mouse; Wellcome Reagents Ltd., MF08) or rabbit anti-mouse (Behringwerke). To avoid false positive reactions, conjugated antisera were absorbed with insolubilized human immunoglobulin and centrifuged to remove aggregates (Zola, 1977a).

Cytotoxic antibody test. Cytotoxicity was titrated by ^{51}Cr release (Sanderson, 1964) in microtitre plates using rabbit complement. Supernatants were collected using the Titertek supernatant harvesting system (Flow Laboratories).

B cell markers. Direct immunofluorescence for surface membrane immunoglobulin (SMIg) was carried out as described previously (Zola, 1977a) using polyvalent antiserum against human immunoglobulin (Wellcome Reagents Ltd., MF01) or anti-serum against human IgM (Behringwerke). Lymphoid cells from blood or tissue were incubated at 37°C for 30 min and washed before testing, to remove adsorbed immunoglobulin. Cells bearing receptors for activated C3 were detected using complement coated zymosan particles as described by Mendes, Miki & Peixinho (1974). Cells carrying Fc receptors were detected using antibody-coated erythrocytes as described by Hallberg, Gurner & Coombs (1973). Cells forming rosettes with mouse erythrocytes (mouse E) were detected as described previously (Zola, 1977c).

RESULTS

Production of colonies

A total of fifty-nine colonies was obtained from ninety-six wells in this experiment. Of these, twenty-three colonies reacted with the immunizing cell line by indirect immunofluorescence. Most of these colonies produced antibody which reacted with a variety of lymphoblastoid cell lines of B, T, or null type. However, one colony, HC11, produced antibody which reacted with B cell lines, but not T or null lines. After cloning a subline HC11A was expanded and studied in more detail.

Reactivity of HC11A with lymphoblastoid cell lines

HC11A supernatant was tested by indirect immunofluorescence against a number of B, T and null cell lines (Table 1). Strong positive reactions were obtained with six B lymphoblastoid cell lines. No specificity was observed for either heavy or light chain types. A line known as U226B which is a myeloma cell line producing IgE was negative with the antiserum, as were three T cell lines and three null cell lines. Thus, the antiserum reacts positively with all B cell lines tested except the myeloma line U226B and fails to react with T and null cell lines tested. The antiserum gave positive immunofluorescence on the immunizing cell line down to a dilution of 1/128, and a ^{51}Cr-release cytotoxicity test gave a 50% kill titre of 1/64.

Reactivity of HC11A with non-lymphoid cells

HC11A failed to react with human erythrocytes (immunofluorescence or agglutination), granulocyte (immunofluorescence) or a mammary carcinoma cell line (immunofluorescence). Granulocytes showed some cytoplasmic fluorescence, but this was readily distinguishable from membrane staining. Monocytes, identified either morphologically or by their uptake of latex particles, did not react with HC11A.

Reactivity of HC11A with lymphocytes in the peripheral blood

The reactivity of HC11A with peripheral blood cells from patients with leukaemia (Table 2) and control donors (Table 3) indicated a general agreement between the numbers of cells staining with HC11A and numbers of cells reacting with other B lymphocyte markers. Leukaemias with high numbers of B cells tend to be high by all markers, whilst T or null type leukaemias are low by all markers. There are, however, some interesting exceptions; in particular the leukaemia provisionally diagnosed as prolymphocytic leukaemia in which there were high numbers of B cells by SMIg, Fc receptor and mouse erythrocyte

TABLE 1. Reactivity of HC11A with lymphoblastoid cell lines

Cell line	Type	Light chain*	Heavy* chain	Surface membrane immunoglobulin†	Immunofluorescence† HC11A + Anti-mouse γ
BALM/1	B-ALL	κ	δ, μ	++	++
BALM/2	B-ALL	κ	δ, μ	++ (30%)	++
RPMI 8866	B	Not known	Not known	++	++
RPMI 8392	B	λ	δ, μ	+ (50%)	++
RPMI 1788	B	λ	Not known	+	++
HRIK	B	Not known	μ	+ (61%)	++ (75%)
U226B	Myeloma	–	–	–	–
CCRF/HSB2	T	–	–	–	–
CCRF/CEM	T	–	–	–	–
JM	T	–	–	–	–
Reh	Null	–	–	–	–
KM3	Null	–	–	–	–
NALM/6	Null	–	–	–	–

* From published data.

† Unless percentages are stated the majority of cells (< 90%) stained with the intensity stated.

TABLE 2. HC11A as a B cell marker: comparison with other markers in leukaemic peripheral blood lymphocytes

		Percentage of cells reacting in lymphocyte marker test†				
Patient*	Diagnosis	HC11A	SMIg	C3R	FcR	Mouse E
1	CLL	64	76	47	80	67
2	CLL	75	2	2	34	95
3	Prolymphocytic leukaemia	37	82	4	76	78
4	ALL (T)	13	4	35	n.d.‡	n.d.
5	ALL (null)	4	7	n.d.	53	1
6	ALL (null)	0	1	0	n.d.	n.d.
7	AML	0	6	2	16	5
8	CML/ALL	5	4	3	8	1
9	AML	5	4	4	19	1

* All patients except Nos 7 and 9 had high numbers of circulating leukaemic cells.

† Markers: HC11A: indirect immunofluorescence with HC11A hybridoma; SMIg: surface membrane immunoglobulin using fluorescein conjugated polyvalent anti-human immunoglobulin; C3R:C3 receptor using C3-coated zymosan particles; FcR:Fc receptor using antibody-coated ox erythrocytes; Mouse E: rosettes with papain-treated mouse erythrocytes. Technical details in Materials and Methods section.

‡ n.d. = Not done.

rosettes but low numbers of cells carrying the C3 receptor and an intermediate number reacting with HC11A, and one of the CLLs (number 2), where the majority of cells reacted with HC11A and formed rosettes with mouse E, but failed to react in the other assays. Overall, the results suggest that HC11A is a B cell marker, but that it does not react with the membrane molecules involved in any of the other B cell marker reactions. The antigen detected by HC11A was absent from the majority of normal or leukaemic

TABLE 3. HC11A as a B cell marker: comparison with other markers* in normal peripheral blood lymphocytes

Patient	HC11A	SMIg GAM	SMIg M	C3R	FcR	Mouse E
1	12	11	10	11	12	16
2	19	18	n.d.†	12	20	17
3	15	18	9	9	15	22
4	21	19	8	11	24	17
5	13	12	9	9	19	14
6	10	8	6	12	23	16
7	9	13	6	14	23	20
8	10	8	5	14	22	17
T cell fraction‡	2	< 1		n.d.	n.d.	n.d.
B cell fraction‡	47	69		n.d.	n.d.	n.d.

* Markers: HC11A: Indirect immunofluorescence with HC11A hybridoma; SMIg: surface membrane immunoglobulin using fluorescein conjugated polyvalent (GAM) or μ-chain specific (M) anti-human immunoglobulin; C3R: C3 receptor using C3-coated zymosan particles; FcR: Fc receptor using antibody-coated ox erythrocytes; Mouse E: rosettes with papain-treated mouse erythrocytes. Technical details in Materials and Methods section.

† n.d. = Not done.

‡ Cells fractionated on nylon wool column.

T cells. The results however, do not exclude the expression of the HC11A antigen by a small subpopulation of T cells. In the nylon wool separated fractions, only 47% of the non-T cell fraction stained with HC11A as compared with 69% expressing SMIg, suggesting that HC11A is either reacting with an antigen expressed on some but not all B lymphocytes, or is more easily shed than surface membrane immunoglobulin during fractionation of lymphocytes. Examination of the results on controls shows that the number of cells reacting with HC11A is higher than the number of B lymphocytes detected using an anti-IgM antibody, but generally very similar to the number detected using a polyvalent anti-immunoglobulin.

Double-marker tests

When peripheral blood lymphocytes were treated with HC11A and fluorescein-conjugated anti-mouse immunoglobulin and then tested for rosetting, the results shown in Table 4 were obtained. These results

TABLE 4. HC11A and E rosette reactions: double marker experiments

Treatment*	Per cent of cells reacting: Fluorescence only	Rosette only	Double marker	Unmarked
(1) HC11A+fluorescent anti-mouse γ; (2) Sheep erythrocytes	20	74	0	6
(1) HC11A+fluorescent anti-mouse γ; (2) Mouse erythrocytes	7	2	12	79

* Peripheral blood lymphocytes were reacted with HC11A and anti-mouse immunoglobulin as described under Materials and Methods, except that azide was omitted and incubation periods were extended to 30 min. After washing the cells were rosetted with mouse or sheep erythrocytes.

confirm that T lymphocytes, identified by sheep E rosetting, do not react with HC11A. A 'null' cell population is found expressing neither the T cell marker nor the HC11A antigen. Although most cells which rosette with mouse erythrocytes express the HC11A antigen, a significant number of cells which do not form rosettes with mouse erythrocytes also express the antigen.

Does HC11A react with surface membrane immunoglobulin?

HC11A did not produce a precipitin line when tested against human serum by gel diffusion or immunoelectrophoresis. Absorption of HC11A with insolubilized human immunoglobulin did not affect the reactivity of the serum with BALM/1 cells, whereas absorption of anti-human immunoglobulin (MFO1) under the same conditions completely abolished staining.

One leukaemia (number 2 in Table 2) reacted with HC11A but not with antibody to SMIg, while in another leukaemia (number 3 in Table 2) the reverse was true for a proportion of the cells.

TABLE 5. Capping and co-capping experiments*

First antibody	37°C incubation	Second antibody	Result (fluorescence)
MFO1 anti γ G†	—	—	Uncapped
MFO1 anti γ G	2 hr	—	Capping and patching
MFO1 anti γ G	2 hr	MFO1 anti γ G	Capping and patching
MFO1 anti γ G	2 hr	HC11A‡	Partial capping+bright fluorescence around all cells
HC11A‡	—	—	Bright, uniform
HC11A	2 hr	—	Capping
HC11A	2 hr	HC11A	Capping (no extra membrane fluorescence)
HC11A	2 hr	MFO1 anti γ G	Capping (no extra membrane fluorescence)
HC11A (followed by unlabelled anti-mouse Ig)	2 hr	MFO1 anti γ G	Capping
HC11A (without anti-mouse Ig)	2 hr	Fluoresceinated anti-mouse Ig	Bright, uniform

* Immunofluorescence was carried out as described under Materials and Methods, except that azide was omitted and incubations were extended to 40 min.

† MFO1: Polyspecific anti γ globulin (Wellcome Reagents).

‡ HC11A was always followed by washing and treatment with fluorescein-conjugated anti-mouse immunoglobulin (except where otherwise indicated).

Capping and co-capping experiments on BALM/1 cells were carried out to investigate the relationship between HC11A and SMIg (Table 5). HC11A antibody did not cap unless followed by antiglobulin, whilst SMIg was capped by a single layer of antibody, indicating that the HC11A antigen and SMIg are not the same molecules.

When SMIg was capped and cells were then reacted with HC11A followed by fluorescein-conjugated anti-mouse immunoglobulin, the HC11A was not found in caps. A high intensity of fluorescence was found distributed around the cell, showing that the HC11A antigen does not co-cap with SMIg. However, when HC11A was capped first and SMIg stained later the majority of cells showed capped immunofluorescence, suggesting that SMIg co-capped with the HC11A antigen. If, in the latter experiment, the conjugated anti-mouse globulin was replaced by un-conjugated material, fluorescence (which could now only be due to the anti-human globulin) was still found in caps. Thus, SMIg co-caps with the HC11A antigen, but if SMIg is capped first, the HC11A antigen is still seen uniformly distributed around the membrane.

Relationship of HC11A to other B cell markers

The other recognized markers for B cells are the receptor for complement components (also found on monocytes), the Fc receptor (also found on some null cells and T cells, as well as monocytes), the mouse E rosette receptor, and the Ia antigen (also on null cells).

Marker tests on the cell lines showed: (i) The B cell lines, apart from HRIK, do not form EA rosettes and thus do not express Fc receptors. (ii) C3 receptors, revealed by Zc rosetting are expressed on only a few cells in the lymphoblastoid cell lines. (iii) The Ia antigen is found on the null type ALLs and the null cell lines which do not react with HC11A. (iv) The B lymphoblastoid cell lines give very few rosettes with mouse E, whereas all the cells stain with HC11A. The double-marker experiment described previously (Table 4) shows that in peripheral blood there is a subpopulation of cells which react with HC11A but do not form mouse E rosettes.

The possibility remains that the antibody is detecting one of these receptors, but at a level of sensitivity which differs from that of the other marker assays.

DISCUSSION

A number of different markers can be used to enumerate B lymphocytes in human blood, but they do not all give the same answers and it is difficult to select a method of choice. In principle, the definitive test for a B lymphocyte is the expression of surface membrane immunoglobulin (SMIg). However, this test is probably the most difficult technically to carry out. SMIg is usually detected by the direct fluorescent antibody method (WHO/IARC, 1974; Preud'homme & Labaume, 1975) or using antibody-coated erythrocytes (Haegert & Coombs, 1976). The detection of B lymphocytes by SMIg is associated with a multiplicity of technical problems. Lymphocytes including non-B cells have receptors for the Fc portion of the immunoglobulin molecule and can therefore take up immunoglobulin either from the serum or, during the test, from the antisera, particularly if they contain aggregates or immune complexes. Whilst it may be possible to distinguish passively absorbed immunoglobulin from SMIg synthesized by the cell, the necessity for doing this introduces extra technical complications. It is generally believed that IgG on cell surfaces is passively absorbed, whilst IgM is more likely to be synthesized by the cell, but in a recent examination of several antisera used for detection of SMIg Sundqvist & Wagner (1978) showed that one commercial antiserum described as IgM-specific reacted more strongly with IgG than with IgM. Although awareness of these problems allows a laboratory to obtain consistent data on the number of B cells in peripheral blood using SMIg as a marker, there is considerable disagreement between laboratories using slightly different procedures or antisera.

The detection of cells expressing receptors for activated complement is not subject to the same technical pitfalls (Mendes *et al.*, 1974). However, receptors for complement are also found on monocytes and on a small number of T lymphocytes. Some lymphocytes including B cells have receptors for the Fc portion of immunoglobulin. However, this makes a poor marker for B cells since, depending on the technique used, Fc receptors can be found on B cells (Dickler & Kunkel, 1972) T cells (Moretta *et al.*, 1975), monocytes, and null cells (Clements & Levy, 1979), including L cells (Horwitz *et al.*, 1978). The Ia antigen which is found on B cells is also found on null cells (Schlossman *et al.*, 1976) and monocytes. B cells have been demonstrated to form rosettes with mouse erythrocytes, but technical variations in the assay determine whether the test detects either a subpopulation of B lymphocytes (Stathopoulos & Elliot, 1974; Forbes & Zalewski, 1976) or either the total B lymphocyte population or a larger population including B lymphocytes (Zola, 1977c).

In this situation, any new marker for B lymphocytes is worth evaluating, and a number of xenogeneic antisera against B cell determinants have been described (Greaves & Brown, 1973; Ishii *et al.*, 1975; Brochier *et al.*, 1976; Welsh & Turner, 1976; Goodfellow *et al.*, 1976; Billing *et al.*, 1976; Espinouse *et al.*, 1978). These antisera cross-react to varying degrees with monocytes and null cells and some are specific only by cytotoxicity, a technique not suited to diagnostic enumeration of lymphocytes in blood. It is not possible to make such antisera on a large scale or in a reproducible manner.

The potential advantages of hybridoma antibodies have been outlined in the Introduction. By using

cloned cells to produce the antibody the probability that it is specific for a single marker is much greater than in normal antisera. However, the possibility remains that the supernatant is either a mixture of antibodies or contains antibody which reacts with a determinant found in several different molecules. Whilst recognizing this possibility, the results will be discussed on the basis that a single antigen is involved. The fact that HC11A reacts specifically with only some cells justifies our view that a single antigen or group of related antigens is involved.

The antigen reacting with the monoclonal antibody HC11A appears to be a B cell marker as judged by several criteria. The antiserum reacted with only the B cell lines tested and failed to react with T and null cell lines. In peripheral blood, non-lymphoid cells including monocytes, did not express the HC11A antigen. Amongst the leukaemic cell preparations examined the majority of cells in chronic lymphocytic leukaemias reacted with HC11A, whilst most cells in acute lymphoblastic leukaemias, both T and null, did not. B cell marker studies on peripheral blood from normal donors show a general agreement between the percentages of cells detected by the HC11A antibody and the other B cell markers. The test on purified T cells and the double marker test indicate that normal T cells do not have the antigen. The precise expression of the antigen on non-T cells of peripheral blood cannot be determined with certainty; since we do not have an absolutely reliable B cell marker. The data as a whole suggest that the antigen is found on cells which are identified as B cells by other markers, and on a few other cells, which may be B cells not reacting in the other tests, pre-B cells or null cells. It is interesting that more than 50% of null lymphocytes in peripheral blood differentiate into SMIg-positive B cells in culture (Chess *et al.*, 1975). At least some of the null cells in normal blood did not express the HC11A antigen and the null cell lines and leukaemias tested did not react to the antibody. Thus, the antigen behaves as a B cell marker, to the limits of our present ability to demonstrate this.

The HC11A antigen does not appear to be directly related to any of the known B-cell markers. Although SMIg was capped by HC11A antibody the reverse was not true. This suggests that SMIg may be associated with the HC11A antigen on the cell membrane, but that there is an excess HC11A, free of SMIg. The HC11A antigen, unlike SMIg, could not be capped by a single layer of antibody. HC11A antibody, unlike antibody to SMIg, could not be removed on a human immunoglobulin absorbent. One CLL (number 2 in Table 2) reacted with HC11A but did not express detectable SMIg, while the pro-lymphocytic leukaemia (number 3 in Table 2) contained many cells expressing SMIg but not HC11A. Thus, the HC11A antigen can be distinguished from SMIg by several criteria. The results on cell lines and leukaemic cells serve to distinguish the HC11A antigen from the Fc receptor, C3 receptor, mouse E rosette receptor, and Ia antigen.

In conclusion the antigen reacting with HC11A appears to be a new marker for B lymphocytes and its evaluation in routine diagnostic use is continuing. It offers technical advantages over existing B cell markers and preparation of sufficient material for general use should be feasible, at least technically.

We are grateful to Drs G. Dart, M. Rice, Margaret Leahy, R. Seshadri and Ann Kupa for providing samples and for valuable discussion. This work is supported by research grants from the National Health and Medical Research Council and the University of Adelaide Anti-Cancer Foundation, and D. Brooks is the recipient of a Flinders University Research Scholarship.

REFERENCES

BILLING, R., RAFIZADEH, B., DREW, I., HARTMAN, G., GALE, R. & TERASAKI, P. (1976) Human B-lymphocyte antigens expressed by lymphocytic and myelocytic leukaemia cells. I. Detection by rabbit antisera. *J. exp. Med.* **144**, 167.

BÖYUM, A. (1968) Separation of leucocytes from blood and bone marrow. *Scand. J. Clin. Lab. Invest.* **21**, supplement 97, 1.

BROCHIER, J., ABOU-HAMED, Y.A., GUELIO, J.P. & REVILLARD, J.P. (1976) Study of human T and B lymphocytes with heterologous antisera. I. Preparation, specificity and properties of antisera. *Immunology*, **31**, 749.

BROWN, G., CAPELLARO, D. & GREAVES, M. (1975) Leukaemia-associated antigens in man. *J. Natl. Cancer Inst.* **55**, 1281.

CHESS, L., LEVINE, H., MACDERMOTT, R.P. & SCHLOSSMAN, S.F. (1975) Immunologic functions of isolated human lymphocyte subpopulations. *J. Immunol.* **115**, 1483.

CLEMENTS, P.J. & LEVY, J. (1979) Receptors for IgG (Fc receptors) on human lymphocytes: re-evaluation by multiple techniques. *Clin. exp. Immunol.* **34**, 281.

COOPER, M.D., KEIGHTLEY, R.G., WU, L-Y.F. & LAWTON, A.R. (1973) Developmental defects of T and B cell lines in humans. *Transplant Rev.* **16**, 51.

DICKLER, H.B. & KUNKEL, H.G. (1972) Interaction of

aggregated γ-globulin with B lymphocytes. *J. exp. Med.* **136**, 191.

ESPINOUSE, D., TOURAINE, F., SCHMITT, D. & TOURAINE, J.L. (1978) Human B-lymphocyte heteroantigens: direct evidence for specificities not shared by monocytes. *Clin. exp. Immunol.* **34**, 379.

FORBES, I.J. & ZALEWSKI, P.D. (1976) A subpopulation of human B lymphocytes that rosette with mouse erythrocytes. *Clin. exp. Immunol.* **26**, 99.

GALFRE, G., HOWE, S.C., MILSTEIN, C., BUTCHER, G.W. & HOWARD, J.C. (1977) Antibodies to major histocompatibility antigens produced by hybrid cell lines. *Nature*, **266**, 550.

GOODFELLOW, P., BARNSTABLE, C., JONES, E., BODMER, W.F., CRUMPTON, M.J. & SNARY, D. (1976) Production of specific antisera to human B lymphocytes. *Tissue Antigens*, **7**, 105.

GREAVES, M.F. & BROWN, G. (1973) A human B lymphocyte specific antigen. *Nature*, **246**, 116.

HAEGERT, D.G. & COOMBS, R.R.A. (1976) Immunoglobulin-positive mononuclear cells in human peripheral blood: detection by mixed anti-globulin and direct anti-globulin-rosetting reactions. *J. Immunol.* **116**, 1426.

HALLBERG, T., GURNER, B.W. & COOMBS, R.R.A. (1973) Opsonic adherence of sensitized ox red cells to human lymphocytes as measured by rosette formation. *Int. Arch. Allergy Appl. Immunol.* **44**, 500.

HAMMERLING, G.J., LEMKE, H., HAMMERLING, U., HOHMANN, C., WALLICH, R., RAJEWSKY, K. (1978) Monoclonal antibodies against murine cell surface antigens: Anti-H-2, Anti-Ia and Anti-T cell antibodies. *Curr. Top. Microbiol. Immunol.* **81**, 100.

HORWITZ, D.A., NIAUDET, P., GREAVES, M.F., DORLING, J. & DETEIX, P. (1978) Surface markers and electron microscopy of human blood L cells: a comparison with T and B lymphocytes. *J. Immunol.* **121**, 678.

ISHII, Y., KOSHIBA, H., UENO, H., MAEYAMA, I., TAKAMI, T., ISHIBASHI, F. & KIKUCHI, K. (1975) Characterization of human B lymphocyte specific antigens. *J. Immunol.* **114**, 466.

KOHLER, G. & MILSTEIN, C. (1975) Continuous cultures of fused cells secreting antibody of predefined specificity. *Nature*, **256**, 495.

LEVY, R., DILLEY, J. & LAMPSON, L.A. (1978) Human normal and leukaemia cell surface antigens, mouse monoclonal antibodies as probes. *Curr. Top. Microbiol. Immunol.* **81**, 164.

MENDES, M.F., MIKI, S.S. & PEIXINHO, Z.F. (1974) Combined detection of human T and B lymphocytes by rosette formation with sheep erythrocytes and zymosan-C3 complexes. *J. Immunol.* **113**, 531.

MORETTA, L., FERRARINI, M., DURANTE, M.L. & MINGARI, M.C. (1975) Expression of a receptor for human IgM by human T cells *in vitro*. *Eur. J. Immunol.* **5**, 565.

PREUD'HOMME, J.L. & LABAUME, S. (1975) Immunofluorescent staining of human lymphocytes for the detection of surface immunoglobulins. *Ann. N.Y. Acad. Sci.* **254**, 254.

SANDERSON, A.R. (1964) Cytotoxic reactions of mouse isoantisera. *Br. J. exp. Pathol.* **45**, 398.

SCHLOSSMAN, S.F., CHESS, L., HUMPHREYS, R.E. & STROMINGER, J.L. (1976) Distribution of Ia-like molecules on the surface of normal and leukemic human cells. *Proc. Natl. Acad. Sci. USA* **73**, 1288.

STATHOPOULOS, G. & ELLIOTT, E.V. (1974) Formation of mouse or sheep red blood cell rosettes by lymphocytes from normal and leukaemic individuals. *Lancet*, **i**, 600.

SUNDQVIST, K.G. & WAGNER, L. (1978) Influence of specificity of reagents and of behaviour of cell surface Ig on the detection and enumeration of immunoglobulin-bearing lymphocytes in humans. *J. Immunol. Methods*, **21**, 229.

TRIZIO, D. & CUDKOWICZ, G. (1974) Separation of T and B lymphocytes by nylon wool columns: evaluation of efficacy by functional assays *in vivo*. *J. Immunol.* **113**, 1093.

WHO/IARC (1974) Workshops: Special Technical report. Identification enumeration and isolation of B and T lymphocytes from human peripheral blood. *Scand. J. Immunol.* **3**, 521.

WELSH, K.I. & TURNER, M.J. (1976) Preparation of antisera specific for human B cells by immunization of rabbits with immune complexes. *Tissue Antigens*, **8**, 197.

ZOLA, H. (1977a) Preparation of antisera with specificity for human T lymphocytes. *Transplantation*, **23**, 222.

ZOLA, H. (1977b) Antisera with specificity for human T lymphocytes. Preparation by selective suppression of the antibody response against shared antigens. *Transplantation*, **24**, 83.

ZOLA, H. (1977c) Fractionation of human lymphocytes using rosette formation with papain-treated mouse erythrocytes. *J. Immunol. Methods*, **18**, 387.

Note added in proof: the hybridoma HC11A has been renamed FMC1 in order to institute a rational numbering system for hybridomas prepared in this laboratory.