Clin. exp. Immunol. (1974) **16**, 541–552.

SURFACE IMMUNOGLOBULINS IN CHRONIC LYMPHATIC LEUKAEMIA, MACROGLOBULINAEMIA AND MYELOMATOSIS

W. KNAPP*, HENRICA R. E. SCHUIT, R. L. H. BOLHUIS† AND W. HIJMANS

Institute for Experimental Gerontology of the Organization for Health Research, TNO, Rijswijk-ZH, The Netherlands

(*Received* 29 *August* 1973)

SUMMARY

The direct method of immunofluorescence was applied for the detection of surface immunoglobulins (Ig) on lymphocytes, obtained from bone marrow and peripheral blood, especially from patients with paraproteinaemia. The results in chronic lymphatic leukaemia (CLL), in the macroglobulinaemia of Waldenström (MW), and in multiple myeloma (MM) show three different patterns as far as the relationship between cytoplasmic and membrane Ig is concerned. In CLL a monoclonal proliferation of cells with surface Ig was found, but the percentage of cells with cytoplasmic fluorescence was normal. In MW the further differentiation in Ig-secreting cells seems to be intact. In MM the percentage of lymphocytes in the peripheral blood with membrane-bound Ig was within normal limits or decreased.

These data suggest that MM can be considered as a neoplasia of already differentiated Ig-secreting cells, localized in the bone marrow. The combined information again indicates the central role of human bone marrow for antibody production.

INTRODUCTION

Until recently studies of the humoral immune response and its deviations have been mainly limited to intracellular immunoglobulins (Ig) and to serum Ig and antibodies. During the last 2 years a number of investigations have reported on the presence of Ig bound to the surface of a sub-population of lymphocytes. (Klein *et al.*, 1970; Pernis, Forni & Amante, 1970; Rabellino, Colons, Grey & Unanue, 1971; Raff, Sternberg & Taylor, 1970). These lymphocytes with easily detectable membrane Ig are considered to be bone marrow-derived precursors of Ig-secreting plasma cells.

An investigation of these cells may yield information to which extent earlier stages are

* Fellow of the Medical Council of Europe, Institute for Immunology, University of Vienna, Vienna, Austria.

† Research Fellow, Rotterdam Radio-Therapy Institute, Rotterdam, The Netherlands.

Correspondence: Dr W. Hijmans, Institute for Experimental Gerontology of the Organization for Health Research, TNO, 151 Lange Kleiweg, Rijswijk-ZH, The Netherlands.

already involved in the abnormal process which finally leads to the appearance of M components (paraproteins). This information may also help to elucidate the kinetics of Ig-producing cells. Previous studies using the immunofluorescence technique for the detection of intracellular immunoglobulins revealed a striking similarity of the distribution profile of human bone marrow cells positive for the different Ig classes and L chain types with the percentage distribution in serum, if corrected for pool size and metabolic rate. This was found in patients with and without paraproteins (Hijmans, Schuit & Hulsing-Hesselink, 1971). Further investigations showed that this bone marrow pattern does not express itself in the distribution of the cytoplasmic positive cells in the peripheral blood in the samples without paraproteinaemia and in IgG paraproteinaemia. In IgM paraproteinaemia the blood pattern is far less pronounced than in the bone marrow and in IgA paraproteinaemia it approaches the bone marrow pattern (Hijmans & Schuit, 1972). These findings led to the suggestion that the initial phase of antibody production takes place in the peripheral lymphoid organs with a homing tendency of the Ig-secreting cells in the bone marrow.

In this paper data obtained with immunofluorescence for membrane-bound Ig on lymphoid cells will be presented. They can be interpreted as to support the previous conclusion.

MATERIALS AND METHODS

Selection of patients

Blood samples and bone marrow specimens were obtained from various hospitals. The indication to perform a biopsy was made by the attending physician. In total 110 persons were investigated.

Diagnostic criteria

A chronic lymphocytic proliferation consisting mainly of mature small lymphocytes with compatible morphology in bone marrow and blood smears was the main criterion for the diagnosis of chronic lymphatic leukaemia (CLL). The diagnosis of multiple myeloma (MM) was made only when the cytology was definite and an identifiable M component was present in the serum. Minimal requirements for the diagnosis of the macroglobulinaemia of Waldenström (MW) were the typical cytological pattern, consisting of lymphoid cells with a granular chromatin structure, usually accompanied by mast cells and plasma cells, and the presence of a homogeneous IgM M component in the serum. These diagnoses were only accepted if they were in agreement with the clinical data.

Preparation of lymphocyte suspensions

Lymphocytes from peripheral blood anticoagulated with EDTA were obtained by gradient centrifugation using the Isopaque–Ficoll technique (Boyum, 1968). Four millilitres of undiluted blood was layered over 2 ml of an Isopaque–Ficoll mixture and centrifuged for 10–15 min at 3000 rpm in a swing-out centrifuge at room temperature. Cells at the interface were aspirated and washed twice with 30 ml of phosphate-buffered saline (PBS). Usually 80–95% of them were small lymphocytes.

Conjugates

Anti-human IgG conjugated with FITC (lot number 871) and anti-human IgA FITC (lot number 6-171) were supplied by Nordic Immunological Laboratories, Tilburg, The

Netherlands. Anti-human IgM was obtained by repeated absorptions of a commercially obtained anti-human Ig serum (Roboz, lot number 31/35) with different immunoglobulin classes. Antisera against Bence Jones proteins of both kappa and lambda type were raised in rabbits.

In later experiments we used conjugated antibodies absorbed with and eluted from solid immunoadsorbents. The immunization and conjugation procedure was performed essentially as described previously (Hijmans, Schuit & Klein, 1969). Tetramethyl rhodamine isothiocyanate (TRITC) and fluorescein isothiocyanate (FITC), both from Baltimore Biological Laboratories (Maryland), were used in these experiments.

All antisera were tested for monospecificity on monoclonal bone marrow cells as described previously (Hijmans *et al.*, 1969). It appeared however that for specific membrane fluorescence the requirements for the degree of purity of the reagents are even more stringent than for specific cytoplasmic fluorescence. Therefore all conjugates were tested for specificity also within the system of membrane fluorescence with thymocytes as negative controls and lymphocytes of patients with CLL as positive controls for the μ chain and the light chains. The other conjugates were assessed against these findings.

Staining technique

Immunofluorescence staining of lymphocytes by the direct technique was performed by incubating $1–3 \times 10^6$ viable lymphocytes suspended in 0·05 ml PBS with 0·05 ml of conjugate at 4°C for 30 min. After incubation the cells were washed twice with PBS at 4°C and resuspended in one to two drops of PBS. One drop was placed on a slide with a coverslip. For counter-staining the cytoplasmic Ig sedimentation chamber slides were prepared as described previously (Hijmans *et al.*, 1969), fixed for 5 min in acetone at −20°C, washed and stained with conjugates of the same specificity, but now labelled with the other fluorochrome.

Fluorescence microscopy

Epi-illumination (Ploem, 1967) with narrow band excitation for the two wavelengths method (Schuit, 1970; Hijmans *et al.*, 1971; Ploem, in preparation) was used as a standard procedure in all studies. An HBO 100 lamp served as a light source and the slides were viewed with a 100x oil immersion objective. Each microscope field was examined for fluorescence and also in phase contrast for cell morphology.

RESULTS

The results from forty-two normal individuals served as a basis for our further investigations and these are summarized in Table 1. In cord blood we found for the three major Ig classes

TABLE 1. Mean percentage of membrane positive peripheral lymphocytes of normal individuals at different ages

Age	Number	μ	γ	α	κ	λ
Cord blood	17	15 (6–24)	8 (0–22)	4 (0–9)	15 (5–35)	8 (2–13)
1–14 years	5	9 (4–23)	5 (1–6)	1 (0–2)	11 (7–15)	4 (2–10)
20–50 years	20	9 (4–16)	5 (1–12)	< 1 (0–7)	10 (4–20)	5 (2–12)

The figures in brackets represent the ranges.

a somewhat higher percentage of positive cells than in adults and also the intensity of the membrane fluorescence as judged by eye was definitely more intense. In agreement with some data in the literature (Preud'homme & Seligmann, 1972a; Siegal, Pernis & Kunkel, 1971; Fröland & Natvig, 1972; Aisenberg & Bloch, 1972) but in variance to others (Grey, Rabellino & Pirofsky, 1971; Papamichail, Brown & Holborow, 1971; Gajl-Peczalska, Biggar, Park & Good, 1972; McLaughlin, Wetherly-Mein, Pitcher & Hobbs, 1973; Piessens *et al.*, 1973) we found a preponderance of IgM in all three age groups.

The results obtained with forty-four persons with an M component in the serum will be presented according to the clinical diagnosis.

TABLE 2. Membrane-bound Ig on peripheral lymphocytes in chronic lymphatic leukaemia

Number	White blood cells ($\times 10^3$)	Percentage lymphocytes	Treatment		Paraprotein	Percentage membrane positive for:
			Cytostatic	Corticosteroid		
1	N.t.p.	N.t.p.	—	—	—	100% μκ
2	110	95	+	+	IgM	100% μκ
3	70	100	+*	+*	—	> 90% μκ
4	39	92	+*	—	n.t.	44% μκ
5	9	85	+*	+	—	21% μκ
6	110	95	+	+	n.t.	55% μκ
7	52	98	+	+	n.t.	67% μκ
8	17	74	+*	+	IgM**	38% μκ
9	120	97	+	+	—	30% μκ
10	24	70	−	−	IgG**	> 90% μλ
11	58	94	+	+	IgM, IgG**	100% μλ
12	17	85	−	−	n.t.	63% μλ
13	=	=	−	+	IgM**	< 90% μλ
14	21	80	−	−	—	76% μλ
15	148	90	−	−	n.t.	81% μ†
16	76	56	−	−	n.t.	69% μ†
17	30	70	+*	−	IgM	> 90% μ†
18	29	78	−	−	n.t.	> 90% κ‡
19	47	90	+*	−	—	> 90% λ‡
20	40	80	−	−	—	Negative §
21	430	100	−	−	IgG**	Negative §
22	57	95	−	−	—	Negative §
23	N.t.p.	N.t.p.	+	+	IgG**	Negative §
24	110	90	+	+	n.t.	Negative §
25	5	30	−	+	n.t.	Negative §
26	73	N.t.p.	−	−	n.t.	Negative §

* No treatment at time of investigation.

† Membrane fluorescence for both L chains was dubious.

‡ Lymphocytes were tested also for IgD and IgE and found to be negative.

§ Some positive cells were occasionally found with a maximum of 4%.

** IgM is not normal, the abnormality might mean a paraprotein of low concentration.

N.t. = not tested.

N.t.p. = no test performed at date of investigation.

Chronic lymphatic leukaemia

Twenty-six patients with CLL were tested for the presence of membrane-bound immunoglobulins (Table 2). The sera of fifteen of these patients were also investigated for the presence of an M component. In three cases a clear-cut M component was found. In five cases heterogenicity was restricted, but a conclusion on a monoclonal reaction was not justified. As far as membrane Ig are concerned no differences between the group with and without an M component could be observed which is in accordance with results of Preud'homme & Seligmann (1972b). In general a strong preponderance of IgM was found. Among our group of twenty-six CLL none showed membrane-bound IgG or IgA, in two cases no H chain could be detected but only kappa and lambda respectively. In seven cases no clear-cut membrane fluorescence with any of the conjugates could be observed. It should be mentioned, however, that lymphocytes in CLL always showed a very weak staining with all conjugates and were never as completely negative as normal lymphocytes or thymocytes are. Either small quantities of all Ig classes are present or these cells have receptors for Ig as suggested by some authors for B cells (Dickler & Kunkel, 1972; Preud'homme & Seligmann, 1972c). Our seven membrane Ig-negative CLL samples were also negative in the rosette test with uncoated SRBC which is considered to be a T-cell marker (Jondal, Holm & Wigzell, 1972; Papamichail & Holborow, 1972; Wybran, Carr & Fudenberg, 1972). A comparable number of membrane Ig-negative CLL has also been reported by McLaughlin *et al.* (1973). In two cases crystalline Ig inclusions of the same class and L chain type as present on the surface could be detected as already described (Hurez *et al.*, 1972; Cawley *et al.*, 1973).

Macroglobulinaemia of Waldenström (MW)

Twenty-eight cases with the diagnosis MW were investigated for the presence of membrane-bound IgM on peripheral lymphocytes. These findings were compared with the relative percentage of bone marrow cells with detectable cytoplasmic IgM (Table 3). Despite the fact that the number of circulating lymphocytes is usually not increased to any extent, the percentage of lymphocytes bearing IgM on the surface is remarkably high and in some cases reaches 100%. There are however also cases with low numbers or even absence of IgM membrane-positive cells. In some cases the distribution of different Ig classes on peripheral lymphocytes is comparable to normal but in others there are obvious deficiencies (Table 4). One gets the impression that cases with a high percentage of peripheral lymphocytes with membrane-bound IgM show also a high percentage of cells with cytoplasmic IgM in the bone marrow. An influence of treatment on the percentage of cells with membrane-bound IgM could not be found.

Multiple myeloma (MM)

In contrast to the findings in CLL and MW the percentage of peripheral lymphocytes with membrane-bound Ig in patients with MM was within normal limits or decreased (Table 5, numbers 2, 10, 12 and 13). In one case (Table 5, number 1) there was an increased number of IgG-carrying cells, but they were of the lambda type of light chain in contrast to the kappa type of this myeloma case. Table 5 also shows the results of membrane fluorescence studies on peripheral lymphocytes in comparison to the distribution of Ig-secreting cells in the bone marrow. In none of the seven cases with IgG, five cases with IgA, and one lambda Bence

TABLE 3. Presence of IgM in the cytoplasm of bone marrow cells and on the surface of peripheral lymphocytes in MW.

Number	Treatment		Cytoplasmic staining of bone marrow cells (relative percentage of stained cells)		Membrane staining of peripheral lymphocytes for IgM (percentage of all lymphocytes)
	Cytostatic	Corticosteroid	μ	κ/λ	
1	−	−	100	> 100	100
2	+	+	100	> 100	100
3	+	−	n.t.	n.t.	100
4	−	−	100	> 100	68
5	−	−	100	> 100	60
6	−	−	95	> 100	52
7	−	−	98	< 0·01	50
8	+	−	57	32	39
9	−	−	100	> 100	36
10	−	−	100	> 100	32
11	+*	+	34	0·1	25
12	+*	+*	91	> 100	20
13	−	−	n.t.	n.t.	20
14	−	−	74	96	16
15	+*	+*	76	84	15
16	−	−	91	49	14
17	+	−	99	> 100	10
18	+	+	97	> 100	4
19	+	−	97	47	4
20	−	−	99	100	3
21	−	−	99	100	3
22	+	−	96	< 0·01	2
23	+	+	95	> 100	2
24	−	−	96	> 100	0
25	−	−	93	> 100	0
26	−	−	90	< 0·01	0
27	+*	+	83	32	0
28	+*	+*	n.t.	n t.	0

*No treatment at time of investigation.

Jones myeloma did the monoclonal bone marrow pattern express itself in the distribution of different Ig classes on peripheral lymphocytes.

The same is true for the limited series of myeloma patients in which we looked for membrane fluorescence in the bone marrow lymphocytes. There is definitely no increase in the IgG and probably not in the IgA-carrying cells. This is in contrast to the figures for MW, which are significantly higher than the number of μ-positive lymphocytes in normal human blood (Table 6).

TABLE 4. Percentage distribution of different Ig classes in bone marrow cells and on peripheral lymphocytes in ten cases of macroglobulinaemia of Waldenström.

Number*	M components	Cytoplasmic staining (relative percentage of stained cells) Bone marrow								Membrane staining (percentage of all lymphocytes) peripheral blood					
		μ	γ	α	δ	ε	$\mu\kappa$	$\mu\lambda$	κ/λ	μ	γ	α	κ	λ	κ/λ
1	IgMκ	100	—	—	—	—	100	—	—	100	—	—	100	—	—
2	IgMκ	100	—	—	—	—	100	—	—	100	—	—	100	—	—
17	IgMκ	99	< 1	< 1	—	—	100	—	—	10	2	< 1	7	11	0·6
20	IgMλ	99	< 1	< 1	—	—	—	100	—	3	12	< 1	13	2	6·5
21	IgMκ	99	< 1	< 1	—	—	100	—	—	2	14	2	17	15	1·1
15	IgMκ	76·1	17·5	6·0	—	0·4	84	16	6·1	15	2	7	24	2	12
11	IgMκ	13·7	36·6	45·4	4·1	0·2	10	90	0·6	25	< 1	11	8	16	0·5
13	IgMλ			n.t.	n.t.	n.t.				20	6	n.t.	n.t.	n.t.	n.t.
3	IgMκ			n.t.	n.t.	n.t.				100	—	—	100	—	—

* Numbers from Table 3.
N.t. = not tested.
— = no positive cells observed.

TABLE 5. Distribution of different Ig classes in the cytoplasm of bone marrow cells and on the surface of peripheral lymphocytes in multiple myeloma

Number	Cytostatic treatment	IEP	Cytoplasmic staining of bone marrow cells (relative percentage of stained cells)						Membrane staining of peripheral lymphocytes (percentage of all lymphocyte)				
			μ	γ	α	δ	ε	κ/λ	μ	γ	α	κ	λ
1	–	IgGκ	< 1	99	< 1	< 1	< 1	> 100	5	27	< 1	10	24
2	+	IgGκ	0	90†	30†	0	0	9·0	12	< 1	< 1	n.t.	n.t.
3	–	IgGλ	0·4	96·1	3·2	0·4	0	0·03	8	8	2	11	4
4	+	IgGλ	0·3	96·1	3·6	0	0	n.t.	14	12	< 1	n.t.	n.t.
5	+	IgGλ				n.t.			4	12	< 1	1	11
6	–	IgGλ				n.t.			12	6	2	16	8
7	+	IgG‡				n.t.			6	7	2	8	9
8	–	IgAκ	0	0	100	0	0	> 100	6	2	0·5	6	2
9	–	IgAκ	0	0	100	0	0	> 100	7	8	< 1	1	11
10	–	IgAκ	< 1	< 1	99	< 1	< 1	> 100	4	< 1	< 1	n.t.	n.t.
11	+*	IgAκ	0	0	100	0	0	> 100	11	1	3	5	< 1
12	+	IgAλ	0	0	100	0	0	< 0·01	2	< 1	1	< 1	4
13	–	λ	Several IgA, some IgM and IgG, nearly 100% λ						3	1	1	5	< 1

* No treatment at time of investigation.
† 20% were positive for γ and α.
‡ L chain type not determined.
N.t. = not tested.

TABLE 6. Results of membrane fluorescence on bone marrow lymphocytes in twelve cases with paraproteinaemia

Paraprotein	Number	Membrane-staining (percentage of all lymphocytes)		
		γ	α	μ
IgG	4	5	—	—
IgA	3	—	4	—
IgM	5	—	—	39

DISCUSSION

The presence of Ig molecules has been demonstrated on the membranes of lymphocytes of several species including man by a variety of techniques (Sell & Gell, 1965; Coombs, Feinstein & Wilson, 1969; Raff *et al.*, 1970; Pernis, Forni & Amante, 1971). The source of those lymphocytes that bear easily detectable immunoglobulins on the surface and those that do not, has been extensively studied in the mouse and chicken (Raff *et al.*, 1970; Unanue *et al.*, 1971; Rabellino & Grey, 1971). These studies clearly showed that the presence of membrane Ig, which is easily detected by direct immunofluorescence, is specific for B lymphocytes. Lack of membrane Ig-positive lymphocytes in some cases of X-linked agammaglobulinaemia (Cooper, Lawton & Bockmann, 1971; Siegal *et al.*, 1971; Froland & Natvig, 1972 a high percentage of Ig-positive cells in Di George syndrome (Gajl-Peczalska *et al.*, 1972) and the absence or low numbers of Ig-positive cells in the normal human thymus support the idea that this is also true for humans. There are indications that the membrane-bound Ig function as antigen receptors (Mäkelä & Cross, 1970; Pierce, Solliday & Asofsky, 1971; Walters & Wigzell, 1970). Reaction of the receptor with an antigen activates lymphocytes, it then induces transformation into blast cells and finally leads both to replication into identical daughter cells and maturation into plasma cells after a series of divisions.

Multiple myeloma, macroglobulinaemia of Waldenström and chronic lymphatic leukaemia are considered to be malignant monoclonal proliferations of bone marrow derived lymphoid cells. Our findings in these three groups clearly show three different immunofluorescence patterns as far as the relationship between cytoplasmic and membrane Ig is concerned. In the majority but not in all cases of CLL a high percentage (usually above 60%) of lymphocytes carry Ig on their membrane of one class and one light chain type with a strong preponderance of IgM. This is in agreement with other reports (Preud'homme *et al.*, 1971, 1972b; Pernis *et al.*, 1971; Grey *et al.*, 1971; Aidenberg & Block, 1972; Fröland & Natvig, 1972; McLaughlin *et al.*, 1973, Piessens *et al.*, 1973). Although a high number of lymphocytes carry membrane Ig the percentage of cells with cytoplasmic Ig is very low in the peripheral blood as well as in the bone marrow (Hijmans & Schuit, 1972). Obviously there is a block in the further differentiation to Ig-secreting cells. Crystalline Ig inclusions in the lymphocytes of two of our patients with CLL and the three cases in the literature (Hurez *et al.*, 1972; Cawley *et al.*, 1973) may represent a side step from the normal differentiation into Ig secreting plasma cells: there is already a cytoplasmic localization but not yet an adequately functioning secretory apparatus.

Waldenström's macroglobulinaemia is similar to CLL as far as membrane Ig are con-

cerned. The number of circulating lymphocytes is usually not increased, but the percentage of lymphocytes bearing IgM on the surface is remarkably high and in some cases reaches 100%. In contrast to CLL however, the further differentiation in MW into Ig-secreting cells seems to be intact. In the bone marrow specimens all differentiation steps can be observed: cells which show exclusively membrane-bound Ig, cells with membrane Ig and cytoplasmic Ig and cells with cytoplasmic but without membrane-bound Ig. The more mature cells of the proliferating clone are located in the bone marrow and only the less differentiated cells appear to circulate, except in one case where we found a high number of cytoplasmic-positive cells in the peripheral blood.

These findings are to some extent in agreement with results reported by Preud'homme *et al.* (1972b). These authors also consider the MW as a leukaemic process with a prepondderant location of mature cells in bone marrow and lymph nodes. Their findings however that treatment with alkylating agents affects primarily those immature cells which do circulate, expressed as a decrease in the percentage of circulating lymphocytes with monoclonal membrane Ig, is not supported by our data. Thirteen of the twenty-eight patients under study had or had had cytostatic treatment. The mean percentage of peripheral lymphocytes with membrane-bound IgM of the treated group was 95% and of the untreated group 84%. This difference is not statistically significant.

In contrast to the findings in CLL and MW the percentage of peripheral lymphocytes with membrane-bound Ig in patients with multiple myeloma was within normal limits or decreased.

The percentage of bone marrow lymphocytes with membrane Ig was never extremely high in these cases and there was no indication for a monoclonal distribution. Our membrane fluorescence data in MM support the hypothesis that MM is a neoplasia of already differentiated Ig-secreting cells localized in the bone marrow. Our data also indicate that is it unlikely that already earlier stages in the differentiation process from B cells to plasma cells are involved in the malignant process. If this would be the case one should expect an abnormal distribution pattern of membrane Ig on lymphocytes in the bone marrow and perhaps also in the peripheral blood.

It has been reported in the literature (Astaldi, Eridani & Ponti, 1968; Boll, 1967; Killmann *et al.*, 1962; Quaglino *et al.*, 1967; Wagner, personal communication) that proliferation of myeloma cells does occur, although the rate of proliferation is low. This in itself can explain the malignant process, but it does not rule out the feeding by another pool of cells. Our investigations have led us to the same conclusion. When comparing the observations in CLL, in MW and in MM, one can conclude that all three diseases are monoclonal proliferations of B cells, the essential difference between them being the localization of the block in the maturation process. These observations are summarized schematically in Table 7. In CLL already early stages are involved and there is a block in the further differentiation. In MW early stages are involved as well, but there is no block in the further differentiation. More differentiated cytoplasmic-positive cells are observed which have a homing tendency in the bone marrow. In MM only later stages of the differentiation process, Ig-secreting cells, are involved.

These data are in agreement with the previous conclusions (Hijmans *et al.*, 1972) that the initial phase of antibody formation, i.e. reaction of the membrane receptors with antigens and activation of these lymphocytes with blast formation takes place in peripheral lymphoid organs with a homing tendency of the Ig-secreting cells in the bone marrow.

TABLE 7. Scheme of the staining patterns in CLL, MW and MM

Disease	Membrane fluorescence peripheral blood	Cytoplasmic fluorescence bone marrow
CLL	+	−
MW	+	+
MM	−	+

ACKNOWLEDGMENTS

We thank Miss P. van den Berg and Dr J. Rádl for the preparation of the conjugates.

REFERENCES

AISENBERG, A.C. & BLOCH, K.J. (1972) Immunoglobulins on the surface of neoplastic lymphocytes. *New Engl. J. Med.* **287,** 272.

ASTALDI, G., ERIDANI, S. & PONTI, G.B. (1968) The proliferative activity of plasma cells from plasmocytoma *in vitro*. *Europ. J. Cancer*, **4,** 9.

BOLL, I. (1967) Die Proliferationskinetik des Plasmocytoms *in vitro* und ihre Beeinflussung durch Stilbamidin. *Blut*, **15,** 82.

BOYÜM, A. (1968) Separation of leukocytes from blood and bone marrow. *Scand. J. clin. lab. Invest.* **1,** 975.

CAWLEY, I.C., BARKER, C.R., BRITCHFORD, R.D. SMITH, J.L. (1973) Intracellular IgA immunoglobulin crystals in chronic lymphocytic leukaemia. *Clin. exp. Immunol.* **13,** 407.

COOMBS, R.R.A., FEINSTEIN, A. & WILSON, A.B. (1969) Immunoglobulin determinants on the surface of human lymphocytes. *Lancet*, **ii,** 1157.

COOPER, M.D., LAWTON, A.R. & BOCKMANN, D.E. (1971) Agammaglobulinaemia with B lymphocytes. Specific defect of plasma-cell differentiation. *Lancet*, **ii,** 791.

DICKLER, H.B. & KUNKEL, H.G. (1972) Interaction of aggregated gamma globulin with B lymphocytes. *J. exp. Med.* **136,** 191.

FRÖLAND, S. & NATVIG, J.B. (1972a) Surface bound immunoglobulin on lymphocytes from normal and immunodeficient humans. *Scand. J. Immunol.* **1,** 1.

FRÖLAND, S. & NATVIG, J.B. (1972b) Class, subclass and allelic exclusion of membrane bound Ig of human B lymphocytes. *J. exp. Med.* **136,** 409.

GAJL-PECZALSKA, K.J., BIGGAR, W.D., PARK., B.H. & GOOD, R.A. (1972) B lymphocytes in di George syndrome. *Lancet*, **ii,** 1344.

GREY, H.M., RABELLINO, E. & PIROFSKY, B. (1971) Immunoglobulins on the surface of lymphocytes. IV. Distribution in hypogammaglobulinaemia, cellular immune deficiency and chronic lymphatic leukemia. *J. clin. Invest.* **50,** 2368.

HIJMANS, W. & SCHUIT, H.R.E. (1972) Immunofluorescence studies on immunoglobulins on the lymphoid cells of human peripheral blood. *Clin. exp. Immunol.* **11,** 483.

HIJMANS, W., SCHUIT, H.R.E. & HULSING-HESSELINK, E. (1971) An immunofluorescence study on intracellular immunoglobulins in human bone marrow cells. *Ann. N.Y. Acad. Sci.* **177,** 290.

HIJMANS, W., SCHUIT, H.R.E. & KLEIN, F. (1969) An immunofluorescence procedure for the detection of intracellular immunoglobulins. *Clin. exp. Immunol.* **4,** 457.

HUREZ, D., FLANDRIN, G., PREUD'HOMME, J.L. & SELIGMANN, M. (1972) Unreleased intracellular monoclonal macroglobulin in chronic lymphatic leukaemia. *Clin. exp. Immunol.* **10,** 223.

JONDAL, M., HOLM, G. & WIGZELL, H. (1972) Surface markers on human B and T lymphocytes. I. A large population of lymphocytes forming nonimmune rosettes with sheep red blood cells. *J. exp. Med.* **136,** 207.

KILLMANN, S.A., CRONKITE, E.P., FLIEDNER, T.M. & BOND, V.P. (1962) Cell proliferation in multiple myeloma studied with tritiated thymidine *in vivo*. *Lab. Invest.* **11**, 845.

KLEIN, E., ESKELAND, T., INOUE, M., STROM, R. & JOHANNSON, B. (1970) Surface immunoglobulin-moieties on lymphoid cells. *Exp. Cell. Res.* **62**, 133.

MÄKELÄ, O. & CROSS, A.M. (1970) The diversity and specialization of immunocytes. *Progr. Allergy*, **14**, 145.

MCLAUGHLIN, H., WETHERLY-MEIN, G., PITCHER, C. & HOBBS, J.R. (1972) Non-immunoglobulin-bearing 'B' lymphocytes in chronic lymphatic leukaemia? *Brit. J. Haematol.* **25**, 7.

PAPAMICHAIL, M., BROWN, J.C. & HOLBOROW, E.J. (1971) Immunoglobulin on the surface of human lymphocytes. *Lancet*, **ii**, 850.

PAPAMICHAIL, M. & HOLBOROW, E.J. (1972) Subpopulations of human peripheral blood lymphocytes distinguished by combined rosette formation and membrane immunofluorescence. *Lancet*, **ii**, 64.

PERNIS, B., FORNI, L. & AMANTE, L. (1970) Immunoglobulin spots on the surface of rabbit lymphocytes. *J. exp. Med.* **132**, 1001.

PERNIS, B., FORNI, L. & AMANTE, L. (1971) Immunoglobulins as cell receptors. *Ann. N.Y. Acad. Sci.* **190**, 420.

PIERCE, C.W., SOLLIDAY, S.M. & ASOFSKY, R. (1972) Immune response *in vitro*. IV. Suppression of primary IgM, IgG and IgA plaque-forming cell responses in mouse spleen cell cultures by class specific antibody to mouse immunoglobulins. *J. exp. Med.* **135**, 675.

PIESSENS, W.F., SCHUR, P.H., MOLONEY, W.C. & CHURCHILL, W.H. (1973) Lymphocyte surface immunoglobulins. Distribution and frequency in lymphoproliferative diseases. *New Engl. J. Med.* **288**, 176.

PLOEM, J.S. (1967) The use of a vertical illuminator with interchangeable dichroic mirrors for fluorescence microscopy with incident light. *Z. wiss. Mikr.* **68**, 129.

PREUD'HOMME, J.L., KLEIN, M., VERROUST, P. & SELIGMANN, M. (1971) Immunoglobulins monoclonales de membrane dans les leucémies lymphoides chroniques. *Eur. J. clin. biol. Res.* **16**, 1025.

PREUD'HOMME, J.L. & SELIGMANN, M. (1972a) Immunoglobulins on the surface of lymphoid cells in Waldenström's macroglobulinemia. *J. clin. Invest.* **51**, 701.

PREUD'HOMME, J.L. & SELIGMANN, M. (1972b) Surface-bound immunoglobulins as a cell marker in human lymphoproliferative diseases. *Blood*, **40**, 777.

PREUD'HOMME, J.L. & SELIGMANN, M. (1972c) Antihuman immunoglobulin G activity of membrane-bound monoclonal IgM in lymphoproliferative disorders. *Proc. nat. Acad. Sci. (Wash.)*, **69**, 2132.

QUAGLINO, D., TORELLI, U., SAULI, S. & MAURI, C. (1967) Cytochemical and autoradiographic investigations on normal and myelomatous plasma cells. *Acta Haemat.* **38**, 79.

RABELLINO, E., COLONS, S., GREY, H.M. & UNANUE, E.R. (1971) Immunoglobulins on the surface of lymphocytes. I. Distribution and quantitation. *J. exp. Med.* **133**, 156.

RABELLINO, E. & GREY, H.M. (1971) Immunoglobulins on the surface of lymphocytes. III. Bursal origin of surface immunoglobulins on chicken lymphocytes. *J. Immunol.* **106**, 1418.

RAFF, M.C., STERNBERG, M. & TAYLOR, R.B. (1970) Immunoglobulin determinants on the surface of mouse lymphoid cells. *Nature (Lond.)*, **225**, 553.

SCHUIT, H.R.E. (1970) Photomicrographic recording of immunofluorescence. *Standardization in Immunofluorescence*. (Ed. by E. J. Holborow), p.159. Blackwell Scientific Publications, Oxford.

SELL, S. & GELL, P.G.H. (1965) Studies on rabbit lymphocytes *in vitro*. I. Stimulation of blast transformation with antiallotype serum. *J. exp. Med.* **122**, 423.

SIEGAL, F.P., PERNIS B. and KUNKEL, H.G. (1971) Lymphocytes in human immunodeficiency states: a study of membrane associated immunoglobulins. *Eur. J. Immunol.* **1**, 482.

UNANUE, E.R., GREY, H.M., RABELLINO, E., CAMPBELL, P. & SCHIDTKE, J. (1971) Immunoglobulins on the surface of lymphocytes. II. The bone marrow as the main source of lymphocytes with detectable surface bound immunoglobulin. *J. exp. Med.* **133**, 1188.

WALTERS, C.S. & WIGZELL, H. (1970) Demonstration of heavy and light chain antigenic determinants on the cell bound receptor for antigen. Similarities between membrane attached and humoral antibodies produced by the same cell. *J. exp. Med.* **132**, 1233.

WYBRAN, J., CARR, M.C. & FUDENBERG, H.H. (1972) The human rosette forming cell as a marker of a population of thymus-derived cells. *J. clin. Invest.* **51**, 2537.