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Rosette formation with mouse erythrocytes. III. Studies in patients with primary immunodeficiency and lymphoproliferative disorders

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SUMMARY

Rosette formation with mouse erythrocytes and other cell-surface markers were examined on lymphocytes from patients with a variety of primary immunodeficiency and lymphoproliferative disorders. Mouse erythrocyte rosette-forming cells and lymphocytes with surface immunoglobulins were regularly absent in patients with Bruton type agammaglobulinaemia, immunodeficiency and thymoma syndrome and severe combined immunodeficiency disease. However, they were present in normal or low numbers in patients with common variable immunodeficiency, selective IgA deficiency and ataxia telangiectasia. Lymphocytes from patients with acute lymphoblastic leukaemia Sézary syndrome and mycosis fungoides made no or few rosettes with mouse erythrocytes. Increased numbers of mouse erythrocyte rosette-forming cells were present in patients with chronic lymphocytic leukaemia and Waldenstrom's macroglobulinaemia. The significance of the mouse erythrocyte rosette as a B-cell marker in the analysis of primary immunodeficiency and lymphoproliferative disorders is discussed.

INTRODUCTION

Surface markers on lymphocytes from patients with various primary immunodeficiency and lymphoproliferative disorders have been extensively studied. These studies have provided a better understanding both of the pathogenesis of these disorders and of some basic defects of the immune response (Cooper *et al.*, 1973; Bentwich & Kunkel, 1973; Seligmann, Preud'Homme & Brouet, 1973; Dickler *et al.*, 1974; Rowe *et al.*, 1973; Gajl-Peczalska *et al.*, 1973; Gupta, 1976). Rosette-formation of MRBC with human lymphocytes has been reported to be a B-cell characteristic (Gupta & Grieco, 1975; Gupta, Good & Siegal, 1975; and Gupta, Good & Siegal, 1976). During ontogeny mouse erythrocyte rosette-forming lymphocytes (MRFC) are present at least as early as surface IgM (Gupta *et al.*, 1976b). In this communication we present a study of MRFC along with other cell surface markers on lymphocytes from patients with a variety of primary immunodeficiency and lymphoproliferative disorders.

MATERIALS AND METHODS

Patients with various primary immunodeficiency and lymphoproliferative disorders were selected from the Immunology, Dermatology and Haematology Clinics of The Memorial Hospital. The patients with primary immunodeficiency disorders were classified according to WHO recommendations (Cooper, Lawton & Bockman, 1971), and included patients with Bruton type agammaglobulinaemia (nine), common variable immunodeficiency (fifteen), immunodeficiency with thymoma (two), selective IgA deficiency (three), ataxia-telangiectasia (two), and severe combined immunodeficiency (five). Patients with lymphoproliferative diseases included chronic lymphocytic leukaemia (nine), Waldenstrom's macroglobulinaemia (one), acute lymphoblastic leukaemia (five), Sézary syndrome (one) and mycosis fungoides (one).

Isolation of mononuclear cells. Mononuclear cells were isolated from heparinized blood on Ficoll–Hypaque density gradients (Böyum, 1968). Mononuclear cells were washed three times in Hanks's balanced salt solution (HBSS) and resuspended to a

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concentration of 5×10^6 cells/ml. Phagocytic cells were labelled by ingestion of latex particles in the presence of 20% autologous plasma. Almost all cells were viable as tested by trypan blue dye exclusion.

Mouse erythrocyte rosettes (MRFC). Mouse erythrocyte rosette-forming lymphocytes were determined by a technique described by Gupta, Good & Siegal (1976). Fifty μl of lymphocyte suspension were mixed with 25 μl of foetal calf serum (heat-inactivated and absorbed with MRBC) and 100 μl of freshly prepared 1% MRBC. This mixture was centrifuged at 200 *g* for 5 min followed by incubation at 28°C for 1 hr. The pellet was gently resuspended and 200 lymphocytes were counted for rosette formation.

Surface immunoglobulins. Surface Ig on lymphocytes were determined essentially as described by Siegal, Pernis & Kunkel (1971). A polyvalent rabbit anti-human immunoglobulin antiserum (PV) and an antiserum monospecific for μ determinants conjugated with fluorescein or tetramethylrhodamine isothiocyanate were used. Lymphocytes ($0{\cdot}5$–1×10^6/tube) were suspended in cold 2% bovine serum albumin in phosphate-buffered saline (BSA/PBS) containing 0·02% sodium azide. Staining was carried out at 4°C for 30 min. Cells were washed three times in BSA/PBS and resuspended in a very small volume. Wet mounts were prepared and 200–300 lymphocytes were counted with a Leitz Ortholux microscope equipped with vertical fluorescence illumination.

Receptors for IgG Fc (aggregated IgG). Aggregated human IgG was prepared by a modification of the technique of Dickler & Kunkel (1972). Lymphocytes ($0{\cdot}5$–1×10^6/tube) in BSA/PBS were incubated with 25 μl of unconjugated heat-aggregated IgG at 4°C for 30 min. Cells were washed in BSA/PBS and stained with fluorescent polyvalent anti-human immunoglobulin antiserum by incubating at 4°C for another 30 min. Cells were washed three times in BSA/PBS and wet mounts were prepared. Two hundred to 300 lymphocytes were counted.

C3 receptors (EAC rosettes). One hundred μl of SRBC coated with 19S antibody against SRBC (Cordis Lab., Miami, Florida) and fresh AKR mouse serum as a source of complement (EAC) were mixed with 100 μl of lymphocyte suspension. The mixture was incubated at 37°C for 30 minutes. SRBC coated with 19S antibody without complement (EA) were run simultaneously as negative controls. The mixture was vigorously resuspended and 200 lymphocytes were counted for rosette formation.

Sheep erythrocyte rosettes (SRFC). T lymphocytes were identified by their spontaneous rosette-formation with SRBC. One hundred μl of lymphocyte suspension were mixed with 25 μl of human AB serum (heat-inactivated and absorbed with SRBC) and 100 μl of 0·5% SRBC. The mixture was then incubated at 37°C for 5 min and centrifuged at 50 *g* for 5 min followed by incubation at 4°C for 18 hr. The pellet was gently resuspended and 200 lymphocytes were counted for rosette-formation.

RESULTS

Primary immunodeficiency diseases

Bruton type agammaglobulinaemia. Results of cell-surface markers in nine patients with Bruton type agammaglobulinaemia are shown in Table 1. All the patients had less than 0·5% lymphocytes with surface immunoglobulins and receptors for MRBC (MRFC). T lymphocytes were within the normal range in all except one (An) patient who had a high proportion of T lymphocytes. Lymphocytes with C3 receptors were normal.

TABLE 1. Lymphocyte subpopulations in patients with Bruton type agammaglobulaeminia

Subjects	SRFC (%)	EAC rosettes (%)	MRFC (%)	Surface immunoglobulins (percent) PV	M
Ma	79·0	11·0	0·2	0·0	0·0
Ra	84·5	7·5	0·0	0·5	0·0
Ri	80·0	5·5	0·5	0·0	0·0
Ia	84·5	3·5	0·0	0·5	0·0
H	91·0	5·5	0·0	0·5	0·0
An	96·5	12·5	0·3	0·0	0·0
BeG	82·0	—	0·3	0·0	0·0
BeD	81·5	—	0·5	0·0	0·0
Ha	83·0	—	0·5	0·0	0·0
Control mean	81·0	12·1	7·4	17·6	6·4
(Range)	(66·5–92·0)	(4·5–22·5)	(2·0–18·0)	(6·0–30·0)	(1·5–17·0)

Common variable immunodeficiency (CVI). Lymphocyte subpopulations in fifteen patients with CVI are shown in Table 2. MRFC and lymphocytes with surface immunoglobulins were seen either in normal or low number. Only two patients had low percentage of MRFC; one of these also had low percentage of lymphocytes with surface immunoglobulin. T lymphocytes were normal in all the patients. Eleven patients with CVI had panhypogammaglobulinaemia and four (Di, Dia, Mro, Sc) had low IgG and IgA and normal or high serum IgM. Patient Di also had cerebellar degeneration of unknown etiology. He had no telangiectasia, or other features of ataxia-telangiectasia.

Selective IgA deficiency and ataxia telangiectasia. Results of the study of surface markers in three patients with selective IgA deficiency and two patients with ataxia telangiectasia (without IgA deficiency) are presented in Table 3. All patients with selective IgA deficiency had normal numbers of MRFC and lymphocytes with surface immunoglobulins. Patient Fr also had lymphosarcoma of the nasopharynx. At the time of study, this patient was not receiving any chemotherapy.

TABLE 2. Lymphocyte subpopulations in patients with common variable immunodeficiency

Subjects	SRFC (%)	MRFC (%)	Surface immunoglobulins (%) PV	M
1. Mc	81·0	8·0	20·0	11·3
2. Sc	87·5	10·0	10·5	—
3. Ed	91·0	5·0	5·0	4·0
4. Co	86·4	5·0	11·5	7·0
5. Ch	89·5	2·0	2·0	1·5
6. Ro	74·5	2·0	10·0	—
7. Ba	78·0	5·5	9·5	—
8. Mro	73·5	2·5	19·0	—
9. Gw	80·0	1·0	16·4	7·7
10. La	85·0	1·5	1·0	—
11. Di	89·0	2·5	13·5	10·0
12. En	82·5	5·5	26·5	6·0
13. Dia	85·5	8·0	18·0	11·0
14. Ca	97·0	3·0	6·0	5·0
15. Cam	86·0	2·0	6·0	6·0
Control mean (Range)	81·0 (66·5–92·0)	7·4 (2·0–18·0)	17·6 (6·0–30·0)	6·4 (1·5–17·0)

TABLE 3. Lymphocyte subpopulations in patients with ataxia telangiectasia (AT) and selective deficiency of immunoglobulin A (SAD)

Subjects	SRFC (%)	MRFC (%)	Surface immunoglobulins (%) PV	M
Fr (AT)	91·5	10·0	10·0	3·5
Ba (AT)	67·0	10·5	22·5	9·0
Le (SAD)	82·0	8·0	11·0	13·5
Li (SAD)	84·5	6·0	10·0	5·0
Ve (SAD)	20·0	5·0	7·0	—
Control mean (Average)	81·0 (66·5–92·0)	7·4 (2·0–18·0)	17·6 (6–30·0)	6·0 (1·5–17·0)

TABLE 4. Lymphocyte subpopulations in patients with syndrome of immunodeficiency with thymoma

Subjects	SRFC (%)	MRFC (%)	Fc (%) (Agg IgG)	Surface immunoglobulins (%) PV	Surface immunoglobulins (%) M
Ka	88·5	0·0	14·0	0·0	0·0
St	67·5	0·5	22·0	0·0	0·0
Control mean (Range)	81·0 (66·5–92·0)	7·4 (2·0–18·0)	16·7 (5·5–37·0)	17·6 (6·0–30·0)	6·4 (1·5–17·0)

TABLE 5. Lymphocyte subpopulations in patients with severe combined immunodeficiency disease

Subjects	SRFC (%)	MRFC (%)	Surface immunoglobulins (%) PV	Surface immunoglobulins (%) M
Ca*	93·0	0·0	0·0	—
Ru*	49·5	0·0	0·0	0·0
Cam†	73·0	11·0	26·0	18·5
Mar‡	50·0	10·5	12·0	12·5
Wi‡	30·5	23·5	70·0	56·0
Control mean (Range)	81·0 (66·5–92·0)	7·4 (2·0–18·0)	17·6 (6·0–30·0)	6·4 (1·5–17·0)

* Partially reconstituted.
† Fully reconstituted (post bone marrow transplant).
‡ SCID with (B cells) partially reconstituted.

Immunodeficiency and thymoma. Two patients with immunodeficiency and thymoma were studied. Results are shown in Table 4. Almost no MRFC or lymphocytes with surface Ig were seen. T lymphocytes and lymphocytes with IgG Fc receptors were normal in number. Patient Ka had panhypogammaglobulinaemia and patient St had low IgG, normal IgA and high IgM. Ka had recurring thymoma that has extended beyond the thymus and St had benign thymoma. The tumours of both responded to surgery or radiotherapy, without concomitant change in their immunodeficiency state.

Severe combined immunodeficiency. Five patients with severe combined immunodeficiency (SCID) disease were studied. Three patients had very low numbers of both T and B cells to begin with, whereas two patients (Mar & Wi) had high proportion of B cells. The studies shown in Table 5 were carried out following bone marrow or organ transplantation. Patient Cam was fully reconstituted and had normal numbers of lymphocytes with surface Ig and receptors for MRBC and SRBC. Patients Ca and Ru were at the time of study only partially reconstituted. They did not have any lymphocytes with surface Ig or MRFC. T lymphocytes were low in Ru and normal in Ca. The patients with a high proportion of B cells (Mar, Wi) also had low proportion of T cells. MRFC and lymphocytes with surface Ig were normal in patient Mar. There was a significant discrepancy between cells with surface IgM and MRFC in patient Wi.

TABLE 6. Lymphocyte subpopulations in patients with chronic lymphocytic leukaemia

Subjects	SRFC (%)	MRFC (%)	Surface immunoglobulins (%) PV	Surface immunoglobulins (%) M
Az	11·0	84·0	0·0	0·0
Mi	9·0	91·0	90·0	90·0
De	45·0	59·0	—	5·8
Wi	10·0	96·0	9·8	—
Sm	18·5	83·5	0·0	0·0
Bu	9·0	70·5	0·0	0·0
Tr	58·0	50·5	—	52·0
Am	29·0	55·0	92·0	87·0
An	25·0	45·5	75·0	74·0
Wa*	43·5	32·0	68·0	49·0
Control mean (Range)	81·0 (66·5–92·0)	7·4 (2·0–18·0)	17·6 (6·0–30·0)	6·4 (1·5–17·0)

* Waldenstrom's macroglobulinaemia.

TABLE 7. Lymphocyte subpopulations in patients with acute lymphoblastic leukaemia, Sézary syndrome and mycosis fungoides

Subjects	SRFC (%)	MRFC (%)	Surface immunoglobulins (%) PV	Surface immunoglobulins (%) M
Ro	11·0	0·0	1·5	0·0
Mo	5·5	1·0	5·5	—
Ri	99·5	1·0	0·5	—
Au	98·0	0·0	1·0	1·0
Co	83·5	0·0	7·0	1·4
Jo*	91·0	7·0	0·0	0·0
Ki†	99·5	0·0	5·0	0·5
Control mean (Range)	81·0 (66·5–92·0)	7·4 (2·0–18·0)	17·6 (6·0–30·0)	6·4 (1·5–17·0)

* Sézary Syndrome.
† Mycosis fungoides.

Lymphoproliferative disorders

Chronic lymphocytic leukaemia. Results of cell-surface markers in nine patients with chronic lymphocytic leukaemia and one patient with Waldenstrom's macroglobulinaemia are shown in Table 6. All nine patients with CLL had increased numbers of MRFC. Four of nine patients had increased number of lymphocytes with surface Ig. Four patients had no demonstrable surface Ig. All five patients with increased surface Ig had increased IgM-bearing cells. T lymphocytes were low in all patients.

The patient with Waldenstrom's macroglobulinaemia had increased numbers of MRFC and lymphocytes with surface IgM. T cells were low.

Acute leukaemia. Five patients with acute lymphoblastic leukaemia and one each with Sézary syndrome and mycosis fungoides were studied. Results are given in Table 7. Three of five patients with acute lymphoblastic leukaemia had increased numbers of lymphocytes forming spontaneous rosettes with SRBC. MRFC were lacking in three and low in the other two. Lymphocytes with surface Ig were either lacking or few. Patient Jo with Sézary syndrome had no lymphocytes with surface Ig; but had a normal number of MRFC. T lymphocytes were increased. Patient Ki with mycosis fungoides had high numbers of T lymphocytes. MRFC and lymphocytes with surface Ig were almost absent.

DISCUSSION

Our results show that all patients studied with Bruton type agammaglobulinaemia had fewer than 0·5% MRFC and lymphocytes with surface IgM, although lymphocytes with C3 receptors were present in normal numbers. T lymphocytes as determined by rosette-formation with SRBC were normal or moderately increased. Similar results with surface immunoglobulin were reported by other investigators (Hayward & Greaves, 1975; Preud'Homme, Griscelli & Seligmann, 1973; Grey, Rabellino & Pirofsky, 1971; Cooper, Lawton & Bockman 1971; Fröland, Natvig & Berdal, 1971; Cooper & Lawton, 1972, Gajl-Peczalska *et al.*, 1973). However, Siegal, Pernis & Kunkel (1971) found low numbers of immunoglobulin-bearing lymphocytes in three of six affected boys, and others (Luckasen *et al.*, 1974; Geha, Rosen & Merler, 1973) have had similar observations. This may be explained because of Ig bound to lymphocytes through Fc receptors and probably does not represent intrinsic immunoglobulin. Hayward & Greaves (1975) also found less than 0·5% lymphocytes with receptors for EBV (a B-cell marker). Normal numbers of lymphocytes with IgG Fc and/or C3 receptors which lack surface Ig have been found (Wernet *et al.*, 1974; Schiff *et al.*, 1974; Yata *et al.*, 1973; Aiuti *et al.*, 1973; Fröland & Natvig, 1972; Hayward & Greaves, 1975; Luckasen *et al.*, 1974). These results differ somewhat from those of Geha, Rosen & Merler (1973) who found no lymphocytes with complement receptors and from those of Preud'Homme *et al.* (1973), who found no cells with receptors for IgG Fc. Cells with receptors for IgG Fc and C3 which lacked surface immunoglobulins have been interpreted either as immature or defective B lymphocytes in which Ig was not expressed, or as some other ('third population' [Fröland, Wisloff & Michalsen, 1974]) cell population or as monocytes (Hayward & Greaves, 1975). 'Third population' cells can be detected by the Ripley rosette test and appear to be involved in antibody-dependent cell-mediated cytotoxicity (Wisloff, Fröland & Michalsen, 1974). Patients with Bruton type agammaglobulinaemia generally lack true B lymphocytes; however, they appear to have normal numbers and normally functioning 'third population' lymphoid cells. Normal or increased numbers of T lymphocytes seen in our patients have also been reported by other investigators (Yata *et al.*, 1973; Schiff *et al.*, 1974; Hayward & Greaves, 1975).

The distribution of circulating B lymphocytes appears to be quite variable in patients with immunodeficiency and thymoma (Siegal, Pernis & Kunkel, 1971; Cooper, Keightley & Lawton, 1975). Both our patients lacked MRFC and lymphocytes with surface immunoglobulin, but had normal numbers of Fc receptor bearing lymphocytes. One of the patients (Ka) had panhypogammaglobulinaemia and somewhat low responses to mitogens and antigens. The other patient (St) had low serum IgG, normal IgA and increased IgM. Lymphocyte responses to antigens and mitogens were markedly depressed in this subject. Cooper & Lawton (1972) also found deficiencies of B lymphocytes, immunoglobulin-containing cells in lymph nodes and serum immunoglobulin. Siegal *et al.* (1971), Siegal *et al.* (1975) and Wernet *et al.* (1974) studied in detail a patient with immunodeficiency and thymoma who had lymphocytes without surface immunoglobulin but which bound aggregated IgG. Lymphocytes from this patient acquired Ig receptors after a few days in culture medium containing normal serum. This syndrome has been suggested to be an acquired deficiency of stem cells, in which the overgrowth of thymic epithelial cells could represent a compensatory hyperplasia (Jeunet & Good, 1968). Assays of circulating thymopoietin are very much needed because the association of thymoma with this immunodeficiency disease remain a dilemma since the original description in 1954 (Good).

Our present studies of Bruton type agammaglobulinaemia and thymoma with immunodeficiencies

clearly demonstrate the regular absence both of MRFC and lymphocytes with SIgM even though cells with C3 and Fc receptors ('third population') are present in normal numbers. These findings confirm the results from study of normal persons (Gupta *et al.*, 1976) that the MRFC marker is independent both of Fc and C3 receptors.

Two major phenotypic patterns of CVI have previously been reported (Wu, Lawton & Cooper, 1973; Preud'Homme, Clauvel & Seligmann, 1975; Nicod, Gerard and Cruchaud, 1973; Dickler *et al.*, 1974; Schiff *et al.*, 1974; Cooper *et al.*, 1971; Luckasen *et al.*, 1974; Preud'Homme & Seligmann, 1972); Siegal *et al.*, 1971; Grey, Rabellino & Pirofsky, 1971), one large group of patients with CVI who have panhypogammaglobulinaemia and a smaller group with IgG and IgA deficiency in whom IgM is normal or increased. Our patients with CVI had normal or somewhat low numbers of lymphocytes with surface immunoglobulin as well as low numbers of lymphocytes with receptors for MRBC. Lymphocytes with receptors for SRBC (T cells) were usually normal. Preud'Homme *et al.* (1973) reported three patients with common variable hypogammaglobulinaemia of early onset with absent surface immunoglobulin-bearing lymphocytes. The results of MRFC in our patients shows the same kind of variability as do the other markers; the MRFC is useful in confirming the presence of B cells in these patients and these findings contrast to their absence in Bruton type agammaglobulinaemia.

Selective IgA deficiency is the most common immunodeficiency pattern. A maturation defect of IgA producing cells is indicated by an absence of plasma cells producing IgA in the presence of normal or increased numbers of circulating lymphocytes with surface IgA (Cooper *et al.*, 1975). Defective T cells cells have been suggested as a possible underlying basis for selective deficiency of IgA. In support of this postulate, analysis of surface markers has sometimes revealed reduced numbers of T cells (Schiff *et al.*, 1974). Our patients, however, had normal numbers of MRFC and lymphocytes with surface immunoglobulins, as well as normal numbers of T cell and responses to both plant mitogens (PHA, Con A) and a variety of antigens (data not shown).

Neither of the two patients with ataxia-telangiectasia studied here had a deficiency of serum IgA or IgE. MRFC, T lymphocytes and lymphocytes with surface immunoglobulin were normal. Seligmann, Preud'Homme & Brouet (1973) and Gajl-Peczalska *et al.* (1973) found normal or increased numbers of IgA bearing lymphocytes in five patients with ataxia-telangiectasia associated with serum IgA deficiency. Luckasen *et al.* (1974) also found normal numbers of lymphocytes with immunoglobulins; they however found a low number of T lymphocytes. Similarly low numbers of T cells and low responses to PHA were reported in certain patients with ataxia-telangiectasia (Yata *et al.*, 1974). More than 10% of patients with ataxia-telangiectasia develop malignancies, particularly involving the lymphoreticular apparatus (Good, 1974). Our patient Fr had lymphosarcoma of the nasopharynx. Both of our patients had low responses to both mitogens and antigens.

Certain patients with SCID have been shown to have very few lymphocytes in thymus, blood, or peripheral lymphoid tissues and very poor functional immunity. As expected, cell-surface marker studies have often confirmed the general paucity of T and B cells (Cooper *et al.*, 1973; Hayward & Greaves, 1975; Meuwissen, Pollara & Pickering, 1975; Yata *et al.*, 1974). Not all patients with functional combined immunodeficiency have severe lymphopenia. Evaluation of lymphocyte surface markers from several patients has revealed an absence of T cells and the presence of surface Ig on virtually all their cells, although distribution by Ig class may be abnormal (Hayward & Greaves, 1975; Cooper & Lawton, 1972; Preud'Homme *et al.*, 1973; Preud'Homme, 1975). In two of our patients who were studied following partial immunologic reconstitution by bone marrow transplantation, low or normal numbers of T cells were present but MRFC along with surface Ig-bearing cells were absent. MRFC and lymphocytes with surface Ig were present in normal numbers in a patient (Cam) fully-reconstituted immunologically after marrow transplantation. The relationship of MRFC and lymphocytes with surface Ig again confirms that the binding of MRBC is a B cell characteristic. However, one of our two patients with SCID who had high numbers of B cells seemed to reveal a possible dissociation between MRFC and surface Ig.

Lymphocytes from a substantial proportion of patients with ALL lack surface markers of T or B lymphocytes. However, lymphocytes from certain patients with ALL have been reported to have

characteristics of either T or B lymphocytes (Borella & Sen, 1974; Aiuti *et al.*, 1973; Davey & Gottlieb, 1974; Borella & Sen, 1973; Kersey *et al.*, 1973; Chin *et al.*, 1973; Fu, Winchester & Kunkel, 1975. In our study, three of five patients had cells with T-cell characteristics whereas two did not demonstrate any surface markers on most of their lymphocytes. MRFC and surface Ig bearing lymphocytes were almost completely lacking in all the cases. Gupta & Grieco (1975) also reported a lack of receptors for SRBC, MRBC and C3 in their patient with ALL. Chin *et al.* (1973) studied fourteen patients with ALL and reported that most of their patients had T-cell markers (as determined by anti-T cell antiserum). Ferrarini *et al.* (1975) reported two of five patients with ALL having high numbers of lymphocytes with surface Ig. Only one of these two patients had a majority of lymphocytes with Fc receptors. Fu *et al.* (1975) reported in certain patients with ALL the lymphocytes bear the HL-B antigens that are normally present on B-lymphocytes and lacking on most T-lymphocytes according to present definitions.

The Sézary syndrome is characterized by generalized pruritic and pigmented erythroderma, lymphadenopathy and the presence of abnormal leucocytes in the skin infiltrates and in the peripheral blood. Both the large typical Sézary cell and its small cell variant have been shown to have cell markers characteristic of T-lymphocytes as determined by SRBC rosette-formation and susceptibility to cytotoxicity with anti-T cell antiserum and lack surface Ig and receptors of IgG Fc and C3 (Broome *et al.*, 1973; Brouet, Flandrin & Seligmann, 1973; Ferrarini *et al.*, 1975). Most of the circulating lymphocytes of our patient had T-cell characteristics. Another patient with mycosis fungoides also lacked MRFC and lymphocytes with surface Ig, but had very high numbers of T-lymphocytes.

Most of the lymphocytes from the vast majority of patients with CLL have been reported to have surface Ig and receptors for IgG Fc and/or C3 (Preud'Homme & Seligmann, 1972; Ross *et al.*, 1973; Seligmann, Preud'Homme & Brouet, 1973); Siegel, Grieco & Gupta, 1974; Siegel, Grieco & Gupta, 1976; Dickler *et al.*, 1973; Angener, Cohnen & Brittinger, 1973 and Brown *et al.*, 1974). However, a few cases of T cell CLL have also been reported (Dickler *et al.*, 1973; Lillie *et al.*, 1973; Yodoi, Takatsuki & Masuda, 1974; Aisenberg, Bloch & Long, 1973). Up to 20–30% of CLL patients were reported to have a circulating population of lymphocytes which lack detectable surface immunoglobulin but which have either receptors for IgG Fc or C3 or both (Ross *et al.*, 1973). MRFC have been reported in high numbers in almost all CLL patients thus far studied for this marker (Gupta & Grieco, 1975; Stathopolous & Elliott, 1974; Catovsky *et al.*, 1975). Our present study demonstrates high numbers of MRFC even in the blood of CLL patients whose lymphocytes lack detectable surface Ig. Therefore, this marker may be particularly useful in defining the nature of CLL cells on which surface Ig is not readily demonstrable. Patients with Waldenstrom's macroglobulinaemia have increased numbers of B lymphocytes with surface IgM (Seligmann, Preud'Homme & Brouet, 1973). Our patient also demonstrated an increased number of lymphocytes with surface IgM and MRFC. We have earlier reported that most lymphocytes with IgM bind to MRBC (Gupta *et al.*, 1976).

This study of MRFC along with other cell-surface markers in a variety of primary immunodeficiency and lymphoproliferative disorders points up the usefulness of this marker for B lymphocytes. It further demonstrates dissociations among markers for B lymphocytes and confirms that the receptor for MRBC is independent of surface Ig and of receptors for IgG Fc and C3.

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