*Clin. exp. Immunol.* (1979) 35, 286–295.

# Fc receptors for IgG, IgM and IgE on human leukaemic lymphocytes

H. L. SPIEGELBERG & P. M. DAINER *Department of Immunopathology, Scripps Clinic and Research Foundation, La Jolla, California and Department of Medicine and Clinical Investigation Center, Naval Regional Medical Center, San Diego, California, USA*

(*Received* 27 *April* 1978)

SUMMARY

Fc receptors for IgG, IgM, IgE and the cell surface immunoglobulins (SIg) were analysed on lymphocytes from seventeen patients with chronic lymphatic leukaemia (CLL), one with lymphosarcoma cell leukaemia (LSL), two each with hairy cell leukaemia (HCL), acute lymphatic leukaemia (ALL) and Sézary syndrome. Fc receptors for IgG and IgM were detected by rosette formation with ox erythrocytes ($E_O$) sensitized with rabbit IgG ($E_OA_G$) and IgM ($E_OA_M$) anti-$E_O$ antibodies, respectively. Fc receptors for IgE were analysed either with $E_O$ coated with glutaraldehyde coupled complexes consisting of rabbit Fab′ fragments of anti-$E_O$ antibodies and Fc fragments of an IgE myeloma protein ($E_OA_E$), or with aldehyde fixed $E_O$ to which IgE was adsorbed. SIg of classes IgM, IgD, IgG and κ and λ light chain type were detected with $E_O$ coated with complexes consisting of Fab′-anti-$E_O$ and purified $F(ab')_2$ fragments of specific goat antibodies.

Lymphocytes of all patients with CLL, LSL and HCL had Fc receptors for IgG ($65\pm15\%$ $E_OA_G^+$, normal $22{\cdot}0\pm5{\cdot}8\%$). Ten patients had significant numbers of cells with IgM Fc receptors ($37\pm22\%$ $E_OA_M^+$, normal $1{\cdot}2\pm1{\cdot}5\%$) which were detected without overnight culturing of the lymphocytes. Lymphocytes of four patients (two CLL, one LSL, one HCL) had Fc receptors for IgE (22–88% $E_OA_E^+$, normal $1{\cdot}8\pm0{\cdot}7\%$). The cells of three of these four patients were also $E_OA_M^+$. The high numbers of rosetting cells indicated that individual lymphocytes must have carried more than one class of Fc receptors. The lymphocytes of the ALL and Sézary syndrome patients had few Fc receptor positive cells.

Of the seventeen patients with CLL, twelve were $SIgM^+$ and/or $SIgD^+$, only four $\kappa^+$ or $\lambda^+$ and one had no SIg. The cells of the LSL and one of the HCL patients were $SIgM^+$ and $SIgD^+$, whilst the cells of the other HCL patient were SIgG, $\lambda^+$. None of the other patients had more than 10% $SIgG^+$ cells. The ALL and Sézary syndrome patients had low numbers of $SIg^+$ and Fc receptor positive cells.

These data indicate that lymphocytes of patients with B-cell leukaemias can carry different classes of Fc receptors simultaneously; the different classes are found in a decreasing frequency of IgG > IgM > IgE.

## INTRODUCTION

Lymphocytes have been divided into three major groups according to their origin and/or specific cell surface markers. Human thymus derived lymphocytes (T cells) are characterized by cell surface receptors for sheep erythrocytes (E) (Jondal, Holm & Wigzell, 1972). Bone marrow derived lymphocytes (B cells)

Correspondence: Dr H. L. Spiegelberg, Scripps Clinic and Research Foundation, 10666 North Torrey Pines Road, La Jolla, California 92037, USA.

0099-9104/79/0020-0286$02.00 

bear membrane-bound surface immunoglobulin (SIg) (Klein *et al.*, 1970; Pernis, Fornie & Amante, 1971; Unanue *et al.*, 1971). A third, as yet poorly defined, population of lymphocytes have neither E receptors nor SIg and their origin is unknown (Frøland, Wisløff & Michaelsen, 1974). Subpopulations of all three groups bear receptors specific for the Fc fragment of a given class of Ig (Dickler, 1976). T cells can have Fc receptors for either IgM or IgG (Moretta *et al.*, 1975; Gmelig-Meyerling, van der Ham & Ballieux, 1976). Fc receptors for IgG were detected on B cells by analysing the binding of heat aggregated IgG (Dickler & Kunkel, 1972) or rosette formation with rabbit IgG-coated ox red cells ($E_O$) (Hallberg, Gurner & Coombs, 1973). In contrast, Fc receptors for IgG could not be detected on B cells but on third population lymphocytes (null cells) with human red cells coated with human (Ripley) IgG antibodies (Frøland *et al.*, 1974). The Fc receptors for IgG may therefore be different on B cells from those on third population lymphocytes (Lobo & Horwitz, 1976). In addition, subpopulations of B cells have been found to have Fc receptors for IgE (Lawrence, Weigle & Spiegelberg, 1975; Gonzalez-Molina & Spiegelberg, 1977) and for IgM (Ferrarini *et al.*, 1977).

Neoplastic cells from patients with leukaemia also show many of these cell surface markers. Lymphocytes from patients with chronic lymphatic leukaemia (CLL) usually have SIg (Grey, Rabellino & Pirofsky, 1971; Preud'homme *et al.*, 1971; Knapp *et al.*, 1974). Cells of most CLL patients have Fc receptors for IgG (Dickler *et al.*, 1973; Ferrarini *et al.*, 1975) and also many for IgM as well (Pichler & Knapp, 1977; Ferrarini *et al.*, 1977). Because lymphocytes from leukaemia patients have not been analysed for Fc receptors for IgE, we have examined the cells from patients with CLL, acute lymphatic leukaemia (ALL), lymphosarcoma cell leukaemia (LSL) and hairy cell leukaemia (HCL) for their ability to form rosettes with IgE coated ox red cells ($E_O$). Because no homologous IgE anti-ox erythrocyte ($E_O$) antibodies are available, the indicator cells were prepared either by adsorbing an IgE myeloma protein to aldehyde fixed ox erythrocytes ($E_O'$) or by sensitizing $E_O$ with rabbit Fab' anti-$E_O$ fragments to which IgE Fc fragments were coupled with glutaraldehyde. The heavy chain class and light chain type of SIg of the cells were also determined by rosette formation, employing $E_O$ coated with complexes of Fab' anti-$E_O$ and purified $F(ab')_2$ fragments of goat anti-Ig antibodies.

## MATERIALS AND METHODS

*Patients.* Lymphocytes from seventeen patients with CLL, one with LSL and two each with HCL, ALL and Sézary syndrome were studied. Except for the first testing of patients 19 and 20 (Mar, Boy) with HCL, all had been treated with alkylating agents and/or steroids for several weeks or months. Five patients were studied twice or three times, 3 or more months apart. The diagnoses were established by clinical and by laboratory criteria. Controls consisted of healthy laboratory personnel.

*Immunoglobulins and fragments.* Normal human IgG and myeloma proteins of different classes were isolated from serum as previously described (Lawrence *et al.*, 1975). For IgE, Fab and Fc fragmentation, the myeloma proteins (Sha and For) were digested with 1% w/w papain for 2 hr at 37°C (Porter, 1959) and then dialysed without alkylation. The Fab and Fc fragments were isolated by DEAE-cellulose chromatography employing 0·005 M phosphate buffer pH 8·0 for elution of the Fab and 0·035 M buffer for the Fc fragments. As shown by sodium dodecyl sulphate polyacrylamide gel electrophoresis, the Fab fragment was degraded in the Fd portion. The Fc fragment consisted of a single band in unreduced form; however, the reduced Fc fragments showed several bands between 20–30,000 mol. wt.

Purified antibodies to γ, μ, δ, α, κ and λ chains were prepared from specific goat antisera employing immunoadsorbents consisting of myeloma proteins, macroglobulins or κ and λ Bence–Jones proteins coupled to Sepharose-4B (Cuatrecasas, Wilcheck & Anfinsen, 1968). The antibodies were digested with pepsin at pH 3·8 as not all the goat IgG was digested at the usual pH 4·0 (Nisonoff *et al.*, 1960), as shown by Ouchterlony analysis with a rabbit antiserum specific for goat IgG Fc fragments.

Rabbit IgG anti-$E_O$ antibodies were isolated from the pooled antisera of rabbits that had been injected repeatedly i.v. with 10% $E_O$. Rabbit IgM anti-$E_O$ was obtained from rabbits injected with 10% $E_O$ incorporated into Freund's complete adjuvant v/v 1 : 1 and bled 11 days after injection. The globulin fraction was precipitated at a 50% saturated ammonium sulphate concentration and applied on a Sephadex G-200 column equilibrated with acetate buffer, pH 4·0. The acidic buffer was used in order to eliminate contamination of the IgM fraction by IgG aggregates. Sensitized $E_O$ were tested by haemagglutination with goat antisera specific for rabbit IgM and IgG; no cross agglutination was observed indicating that there was no significant contamination of the two antibody preparations. The direct agglutination titres of the two preparations were: 1 : 64 at 20 mg/ml IgG; and 1 : 16 at 7·5 mg/ml IgM, employing 0·2% $E_O$.

The rabbit IgG was digested with pepsin (Nisonoff *et al.*, 1960) and dialysed against PBS. It was then reduced to Fab'

fragments in 0·05 M Tris buffer, pH 8·2, by adding 0·02 M dithiothreitol for 2 hr at room temperature and was alkylated with 0·05 M iodoacetamide. The $F(ab')_2$ fragments had an agglutination titre identical to that of the original IgG fraction. The Fab′ fragments did not agglutinate $E_O$.

*Fab′-F(ab′)₂ and Fab′-Fc IgE coupling.* For use in rosette assays, the purified $F(ab')_2$ anti-Ig fragments and IgE Fc fragments were first coupled to Fab′ anti-$E_O$ with glutaraldehyde (Strausbach, Sulica & Givol, 1970). Of the various concentrations tested, the coupling of 3·0 mg Fab′ anti-$E_O$ with 3·0 mg of either purified $F(ab')_2$ anti-Ig or Fc IgE fragments in a total volume of 1·0 ml PBS, pH 7·4, proved to result in optimal rosette formation. The proteins were brought to 0·9 ml, and 0·1 ml of a fresh 1% glutaraldehyde preparation in $H_2O$ was added and incubated for 1 hr at room temperature. 100 mg of sodium bisulphite was then added to the slightly yellowed solution. After dialysis against PBS, the complexes were stored at −20°C before use.

*Lymphocyte isolation.* Lymphocytes were isolated from anti-coagulated venous blood. The red cells were sedimented for 1 hr at 37°C after adding an amount equal to one half the blood volume of 3% dextran in Tris-buffered Hanks's balanced salt solution. Mononuclear cells were isolated from the supernatant by Ficoll–Hypaque centrifugation (Bøyum, 1968). For the isolation of normal lymphocytes, phagocytic cells were removed before the Ficoll–Hypaque centrifugation by incubating the cells with colloidal iron (Gonzalez-Molina & Spiegelberg, 1977). The yield of lymphocytes was usually about 50%. The isolated lymphocytes were suspended in $5 \times 10^6$/ml Eagle's minimum essential medium (MEM) containing 2·5% heat-inactivated foetal calf serum (FCS).

*Rosette assays.* For demonstration of spontaneous rosette formation with E, 0·1 ml lymphocytes were mixed with 1% E suspended in heat-inactivated FCS and incubated at 37°C for 5–10 min and then overnight at 4°C.

Fc receptors for IgG were detected with rabbit IgG coated $E_O$ ($E_OA_G$) as described previously (Gonzalez-Molina & Spiegelberg, 1977). $E_O$ were sensitized with a 1 : 20 dilution of the rabbit anti-$E_O$ antiserum preparation for 30 min at 37°C for the determination of Fc receptors for IgM ($E_OA_M$). Fc receptors for IgE ($E_OA_E$) were analysed by two methods. First, we used aldehyde-fixed $E_O$ ($E_O'$) to which the IgE myeloma protein Sha had been adsorbed ($E_O'$-IgE) (Gonzalez-Molina & Spiegelberg, 1977). Then fresh $E_O$ were sensitized with complexes of Fab′ anti-$E_O$ fragments and IgE Fc fragments. Maximum sensitization was obtained with 3·0–4·0 mg complexes per ml of 1% $E_O$. Routinely, 0·1 ml of 2% $E_O$ were incubated with 0·5 mg complexes for 1 hr at room temperature. The cells were then washed three times in PBS and stored at 4°C as a 1% solution in MEM for 1 week. Lymphocytes from the HCL patients could not be tested with the $E_O'$-IgE since fixed uncoated $E_O'$ spontaneously formed rosettes with 70% of the cells.

SIg of classes M, D and G and κ and λ light chain type were determined with $E_O$ sensitized with Fab′-anti-ox-$F(ab')_2$ anti-Ig complexes. Optimum rosetting with all antisera was in the same range. Routinely, 0·1 ml of 10% $E_O$ were incubated with 0·1 ml of complexes (6·0 mg/ml) for 1 hr at room temperature, washed and stored as a 1% solution in MEM.

For determination of maximum sensitization, we used various SIg and Fc receptor bearing lymphoblastoid cell lines (Gonzalez-Molina & Spiegelberg, 1976) because they show a large and relatively consistent number of rosette-forming cells with optimally sensitized indicator erythrocytes. Anti-IgG indicator cells did not form rosettes with our lines of cultured lymphoblastoid cells. However, they formed 40–50% rosettes with cells of a line of cultured leukaemic lymphocytes obtained twice from Dr I. Royston, University of California at San Diego. The specificity of the indicator cells was tested by inhibition with myeloma proteins of different classes and with κ and λ Bence–Jones proteins at a final concentration of 1·0 mg/ml.

For all Fc receptors and SIg rosette assays, 0·1 ml lymphocytes were added to 0·1 ml 1% indicator red cells, mixed and centrifuged 5 min at 200 *g*. After incubation for 1–20 hr at 4°C, 100 µl was aspirated and 50 µl 0·05% toluidine blue added to stain the lymphocytes. Higher toluidine blue concentrations caused non-specific adherence of the fixed $E_O$ or $E_O'$-IgE to the lymphocytes. The cells were gently resuspended, and the number of rosettes (a white cell to which at least three red cells adhered) was counted immediately. 300 cells were counted to obtain the percentage of rosetting cells.

Mixed rosette experiments were performed by simultaneously adding fresh and fixed indicator red cells; only the latter became stained with toluidine blue (Gonzalez-Molina & Spiegelberg, 1977). Lymphocytes that bound at least two of each kind of erythrocytes were scored as cells having both markers.

## RESULTS

### *Rosette assays with glutaraldehyde coupled complexes of Fab′-anti-$E_O$ and IgE Fc fragments or $F(ab')_2$ anti-Ig fragments*

Because IgE antibodies to erythrocytes are unobtainable, Fab′ fragments of rabbit anti-$E_O$ antibodies were coupled to Fc fragments of IgE myeloma proteins and used as complexes to sensitize $E_O$ in our rosette assay, as shown in Fig. 1. We developed this method because it is difficult to prepare $E_O'$ coated with IgE which give the same numbers of positive cells in each batch and because uncoated $E_O'$ spontaneously formed rosettes with HCL cells and with 0·5–2% normal lymphocytes. The same method was adapted for the detection of SIg by coupling $F(ab')_2$ fragments of purified goat anti-Ig antibodies to Fab′ anti-$E_O$ (Fig. 1). Purified antibodies were superior to $F(ab')_2$ fragments prepared from the antiserum IgG fraction. The advantages of the anti-SIg rosette tests are the use of a regular light

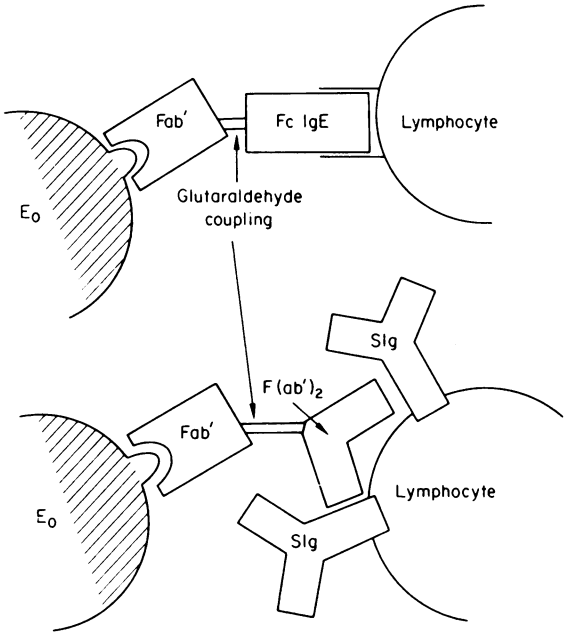


FIG. 1. Schematic presentation of rosette assay employing glutaraldehyde coupled complexes, formed by rabbit Fab' fragments of antibodies to ox erythrocytes ($E_O$) and either Fc fragments of IgE or purified $F(ab')_2$ fragments of anti-human Ig antibodies, for the detection of Fc receptors for IgE and cell surface immunoglobulin (SIg) on lymphocytes, respectively.

microscope and the stability of the Fab'-$F(ab')_2$ complexes. Their sensitivity is equal to immunofluorescence (Hellström, Perlmann & Spiegelberg, 1978).

To standardize this rosette assay, we used cultured human lymphoblastoid cells which contain more rosetting cells than normal lymphocytes and show little variation in the total number of positive cells, regardless of growth phase. The average percentages of rosetting cells between optimally sensitized $E_O$ and different cell lines are shown in Table 1. All lines always contained a significant number of cells that did not rosette. Sub-optimally and over-sensitized red cells formed fewer rosettes (Fig. 2) with cultured lymphocytes and rosetted little or not at all with normal lymphocytes. About ten time more complexes were necessary to obtain optimal sensitization for the Fc IgE receptor detection than for SIg determination (Fig. 2).

TABLE 1. Average percentage of cultured human lymphoblastoid cell lines forming rosettes with optimally sensitized erythrocytes

| | Percentage of rosetting cells | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Surface immunoglobulin | | | | | Fc receptors | | |
| Cell line | SIgM | SIgD | SIgG | κ | λ | $E_OA_G$ | $E_OA_E$ | $E_OA_M$ |
| Wil-2WT | 55 | 75 | 0 | 90 | 0 | 5–15 | 85 | 0 |
| RPMI-8866 | 75 | 0–5 | 0 | 90 | 0 | 0–5 | 90 | 0 |
| RPMI-8392 | 80 | 0 | 0 | 0 | 75 | 15 | 75 | n.t. |
| Raji | 85 | 0 | 0 | 0–1 | 0 | 75 | 0 | 0 |
| Daudi | 65 | 0 | 0 | 85 | 0 | 50 | 0 | 0 |

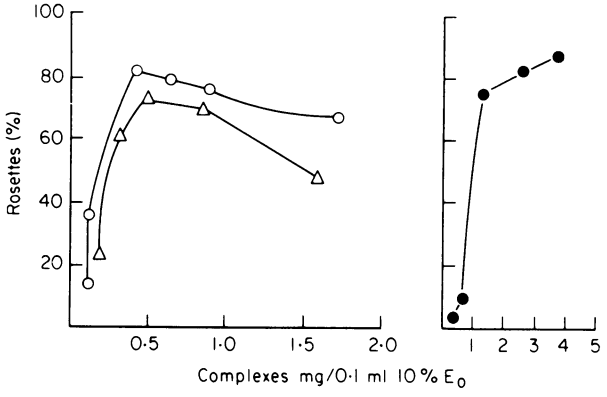


FIG. 2. Titration of rosette formation with $E_O$ coated with glutaraldehyde coupled complexes of rabbit Fab′ anti-$E_O$ and goat $F(ab')_2$ anti-κ ($E_O$-aκ), $F(ab')_2$ anti-IgD chain ($E_O$-aIgD) and IgE Fc fragments ($E_O$-Fc IgE) on cultured human lymphoblastoid cells Wil-2WT.

*Membrane markers on normal lymphocytes*

The surface markers identified on cells from eight normal donors are shown in Table 2. On average, the normals had 6·3% $SIgM^+$, 5·8% $SIgD^+$ and 2·0% $SIgG^+$ cells. The number of $\kappa^+$ cells was 11·5% and $\lambda^+$ cells 8·3%. The total of κ and λ positive cells was always significantly higher than the number of SIgM and SIgD positive cells. The average for cells having Fc receptors were 22% $E_OA_G^+$, 1·8% $E_OA_E^+$ and 1·2% $E_OA_M^+$ lymphocytes. Fc receptors for IgM were analysed routinely on freshly isolated lymphocytes under the same conditions as those used for the leukaemic cells. Few $E_OA_M$ rosetting cells were found; however, after overnight culturing 25–40% of the lymphocytes from three of these donors formed rosettes (not shown in Table 2). The average number of $E^+$ cells was 67·6%.

*Membrane markers on leukaemic lymphocytes*

The surface markers identified on lymphocytes from leukaemic patients are summarized in Table 3. Most of the patients with CLL, LSL and HCL had significant numbers of $SIg^+$ cells, most commonly $SIgM^+$ and $SIgD^+$. Only one patient (No. 20, Boy, HCL) had a relatively high number of $SIgG^+$ cells. Some had large numbers of only light chain positive cells and one patient (No. 15, Hac) had no monoclonal sIg. Usually, one light chain type predominated; however, significant numbers of cells positive for the other light chain type were always found. Both patients with ALL had low percentages of $SIgM^+$

TABLE 2. Surface markers on lymphocytes from eight normal donors

| | Surface immunoglobulin | | | | | Fc receptors | | | T cells |
|---|---|---|---|---|---|---|---|---|---|
| Donor | SIgM | SIgD | SIgG | κ | λ | $E_OA_G$ | $E_OA_E$ | $E_OA_M$ | E |
| 1 Pat | 10·0 | 6·3 | 2·0 | 18·0 | 19·7 | 26·0 | 2·7 | 1·7 | 58·3 |
| 2 Sto | 2·3 | 2·3 | 0·7 | 6·3 | 0·7 | 11·0 | 1·0 | 0·0 | 56·7 |
| 3 Por | 5·5 | 6·0 | 3·7 | 7·3 | 2·8 | 20·3 | 2·3 | 4·3 | 72·3 |
| 4 Spi | 7·5 | 4·5 | 3·5 | 9·0 | 8·0 | 22·0 | 2·5 | 0·3 | 51·7 |
| 5 Fri | 7·7 | 5·3 | 1·0 | 10·3 | 8·3 | 31·7 | 1·5 | 2·3 | 73·0 |
| 6 Wod | 10·3 | 10·5 | 0·3 | 14·7 | 15·0 | 21·0 | 0·7 | 0·3 | 71·0 |
| 7 Bai | 2·7 | 6·3 | 4·0 | 19·0 | 6·7 | 21·7 | 1·7 | 0·0 | 72·0 |
| 8 Bat | 4·5 | 5·3 | 1·0 | 7·8 | 5·0 | 22·0 | 2·0 | 1·0 | 86·0 |
| Average | 6·3 | 5·8 | 2·0 | 11·6 | 8·3 | 22·0 | 1·8 | 1·2 | 67·6 |
| s.d. | ±3·1 | ±2·3 | ±1·5 | ±5·0 | ±6·3 | ±5·8 | ±0·71 | ±1·5 | ±11·2 |

cells, one patient was $E^+$, the other $E^-$. Only few $SIg^+$ cells were found in the Sézary syndrome patients.

All patients with CLL, LSL and HCL had significantly more $E_OA_G^+$ cells than normal, whereas ALL and Sézary syndrome patients were relatively subnormal in this respect. Two CLL, one LSL and one HCL patient had large numbers of $E_OA_E^+$ positive cells. Patient No. 3 (Eck) was tested three times. The first time, when his cell count was 38,000 lymphocytes/mm³, 4% of the cells were $E_OA_E^+$, and the second time, when he had 178,000 cells/mm³, 22% were $E_OA_E^+$. At the third examination, when he had 98,000 cells/mm³, he had 20% $E_OA_E^+$ cells. Ten of the CLL, LSL and HCL patients had high percentages of $E_OA_M^+$ cells in the absence of overnight culturing of the lymphocytes. A significant number of $E_OA_G^+$ cells were found in one of the two Sézary syndrome patients.

Patient No. 18 (Kra) with LSL had higher numbers of $SIgM^+$ than $SIgD^+$ cells. When he was tested the first time, he showed 27% $E_OA_E^+$ cells which could have been restricted to either the $SIgM^+$ or

TABLE 3. Surface markers on lymphocytes from patients with chronic lymphatic leukaemia (CLL), lymphosarcoma cell leukaemia (LSL), hairy cell leukaemia (HCL), acute lymphatic leukaemia (ALL) and Sézary syndrome

| Patient | | WBC/mm³ | Percentage of rosetting cells | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Surface immunuglobulin | | | | | Fc receptors | | | T cells |
| | | | SIgM | SIgD | SIgG | SIgκ | SIgλ | $E_OA_G$ | $E_OA_E$ | $E_OA_M$ | E |
| CLL | | | | | | | | | | | |
| 1 | Lee | 9,200 | 82 | 70 | 0 | 91 | 30 | 85 | 2 | 9 | 11 |
| 2 | O'Ne | 14,200 | 97 | 82 | 2 | 98 | 12 | 74 | 2 | 3 | 10 |
| 3a | Eck | 38,900 | 80 | 85 | 1 | 2 | 86 | 60 | 4 | 1 | 7 |
| 3b | Eck | 178,000 | 61 | 82 | 3 | 32 | 75 | 88 | 22 | 23 | 6 |
| 4 | Han | 51,900 | 80 | 52 | 2 | 32 | 51 | 66 | 2 | 1 | 14 |
| 5 | Hub | 5,200 | 25 | 19 | 2 | 25 | 19 | 30 | 2 | 1 | 59 |
| 6 | Nun | 59,000 | 65 | 25 | 0 | 91 | 3 | 71 | 2 | 82 | 2 |
| 7 | Fri | 55,000 | 64 | 56 | 2 | 89 | 1 | 50 | 0 | 37 | 2 |
| 8 | Pat | 21,000 | 60 | 66 | 2 | 84 | 6 | 72 | 0 | 48 | 8 |
| 9 | Bra | 8,000 | 14 | 24 | 0 | 52 | 14 | 55 | 0 | 0 | 48 |
| 10 | Sim | 98,000 | 75 | 7 | n.t.* | 6 | 71 | 80 | 4 | 37 | 5 |
| 11 | Con | 19,000 | 3 | 57 | 1 | 76 | 8 | 78 | 0 | 5 | 24 |
| 12 | Par | 5,400 | 8 | 25 | 0 | 42 | 22 | 74 | 0 | 2 | 8 |
| 13a | Kin | 14,000 | 6 | 11 | 5 | 54 | 5 | 65 | 0 | 6 | 9 |
| 13b | Kin | 17,300 | 5 | 9 | 8 | 62 | 11 | 54 | 3 | 10 | 8 |
| 14 | Doy | 20,200 | 9 | 4 | 9 | 31 | 52 | 32 | 5 | 38 | 11 |
| 15 | Hac | 40,000 | 5 | 0 | 0 | 19 | 19 | 65 | 82 | 58 | 0 |
| 16 | Mil | 15,000 | 2 | 3 | 0 | 91 | 3 | 75 | 2 | 1 | 13 |
| 17 | Cop | 16,900 | 3 | 7 | 0 | 22 | 68 | 74 | 0 | 0 | 21 |
| LSL | | | | | | | | | | | |
| 18a | Kra | 11,500 | 73 | 42 | 0 | 84 | 4 | 77 | 27 | 10 | 13 |
| 18b | Kra | 15,300 | 65 | 27 | 5 | 63 | 3 | 50 | 46 | 17 | 19 |
| HCL | | | | | | | | | | | |
| 19 | Mar | 11,000 | 53 | 72 | 7 | 78 | 8 | 73 | 37 | 0 | 26 |
| 20 | Boy | 7,000 | 6 | 2 | 23 | 22 | 68 | 53 | 2 | 19 | 10 |
| ALL | | | | | | | | | | | |
| 21 | Sta | 11,000 | 20 | 1 | 0 | 3 | 7 | 6 | 0 | 1 | 11 |
| 22 | McM | 12,000 | 13 | 4 | 2 | 21 | 3 | 16 | 1 | 1 | 53 |
| Sézary | | | | | | | | | | | |
| 23 | Bu | 190,000 | 3 | 0 | 6 | 14 | 10 | 15 | 1 | 2 | 84 |
| 24 | Pa | > 100,000 | 2 | 1 | n.t. | n.t. | n.t. | 5 | 0 | 3 | 64 |

* n.t. = Not tested.

$SIgD^+$ cell populations. We therefore investigated whether the $E_OA_E^+$ cells belonged to a particular type of $SIg^+$ cells by mixed rosetting. As shown in Table 4, mixed rosettes were found with both $SIgM^+$ and $SIgD^+$ cells. More mixed rosettes were found among the patient's $SIgM^+$ cells, the majority of the tumour cells, suggesting that the $E_OA_E^+$ marker is not preferentially associated with either $SIgM^+$ or $SIgD^+$ cells.

The percentage of E-rosetting cells in the CLL, LSL and HCL patients was usually lower than that in normal controls. It was at the lower limit of normal in the patients who had a low overall number of white cells.

## DISCUSSION

These experiments demonstrate that leukaemic lymphocytes from patients with CLL, LSL and HCL can carry either one, two or even all three known classes of lymphocyte Fc receptors. However, the frequency of the three Fc receptor classes differs. Fc receptors for IgG are most common and were demonstrable on cells of all such patients. In contrast, Fc receptors for IgM and for IgE were detected on cells of only 50 and 20% of these patients, respectively. Often, more than 50% of the cells rosetted with different indicator cells, suggesting that many individual lymphocytes must bear two or three different classes of Fc receptors simultaneously. Leukaemic lymphocytes appear to be different in this respect from most normal peripheral lymphocytes. Few normal B cells have Fc receptors for IgG and IgE (Gonzalez-Molina & Spiegelberg, 1977) and T cells usually have Fc receptors for either IgG or IgM, but not both (Moretta *et al.*, 1975; 1977). Even cultured human lymphoblastoid cells, with few exceptions, were found to express mainly one type of Fc receptor (Gonzalez-Molina & Spiegelberg, 1976). In contrast, Fc receptors for IgG and IgM have previously been found to exist simultaneously on human CLL cells (Pichler & Knapp, 1977; Ferrarini *et al.*, 1977) and on murine theta positive lymphoma cells (Neauport-Sautes & Fridman, 1977). It appears, therefore, that the expression of more than one class of Fc receptors is a characteristic of leukaemic lymphocytes. It is unknown whether this state results from neoplastic transformation or if normal immature lymphocytes, like leukaemic cells, also express various Fc receptors. It has been suggested that Fc receptors of B and T cells may be involved in immunoregulation (Kerbel & Davies, 1974), and recent experiments demonstrating that T suppressor cells have Fc receptors for IgG (Moretta *et al.*, 1977; Fridman *et al.*, 1975) and T helper cells for IgM, support this hypothesis. Since leukaemic lymphocytes express more than one Fc receptor, these receptors are probably physiologically non-functional in neoplastically transformed cells. If each Fc receptor has a distinct function then the occurrence of two would tend to have a cancelling effect.

No apparent correlation exists between the expression of Fc receptors and SIg. In confirmation of previous observations (Kubo, Grey & Pirofsky, 1974; Fu, Winchester and Kunkel, 1974; Preud'homme *et al.*, 1974), leukaemic B cells most frequently carried SIgM and SIgD, fewer carried either SIgM or SIgD, light chains only, or no SIg. The light chain type was either κ or λ, as was expected (Levy *et al.*, 1977) for a monoclonal expansion of B lymphocytes; however, in several patients substantial numbers of

TABLE 4. SIg and $E_OA_E$ mixed rosettes formed by lymphocytes from patient Kra with lymphosarcoma cell leukaemia

| Percentage of total rosettes | Percentage of total rosettes | | | Percentage of $E_OA_E$ positive cells that were mixed |
|---|---|---|---|---|
| | SIgM | $E_OA_E$ | Mixed | |
| 70·5 | 84 | 3 | 13 | 81 |
| | SIgD | $E_OA_E$ | Mixed | |
| 23·5 | 59 | 21 | 20 | 49 |

both κ and λ positive cells were found. This probably resulted from the incomplete removal of IgG bound to the Fc receptors, since all cells had these receptors and no special effort was made to wash off the cytophilically bound IgG (Lobo, Westervelt & Horwitz, 1975). Apparently, the anti-light chain indicator cells were particularly sensitive in the detection of cytophilic IgG because the number of $SIgG^+$ cells was always below 10%, except for one patient with HCL. One of the Fc IgE receptor positive patients had a major $SIgM^+$ and minor $SIgD^+$ cell population. In order to determine whether the IgE Fc receptors could have been associated with only one SIg class, mixed rosette analyses were performed. These experiments showed that Fc IgE receptor positive cells were present among both $SIgM^+$ and $SIgD^+$ cells, the numbers of mixed rosettes being approximately proportional to the numbers of $SIgM^+$ and $SIgD^+$ cells. Therefore, cells carrying Fc IgE receptors were probably distributed at random among cells differing in SIg classes. Of the four patients with $E_OA_E^+$ lymphocytes, three were $SIgM^+$ and $SIgD^+$ as are most normal B lymphocytes (Rowe *et al.*, 1973). However, one of these patients had LSL, a rare form of leukaemia, one had HCL and the lymphocytes of one of the two CLL patients with $E_OA_E^+$ cells were $SIg^-$, a rare occurrence for CLL lymphocytes. Normal $E_OA_E^+$ cells appear to be B cells (Gonzalez-Molina & Spiegelberg, 1977); however, the nature and function is unknown. The observation that three out of four patients with $E_OA_E^+$ cells showed uncommon features suggests that the normal lymphocytes that bear Fc IgE receptors may represent a subgroup of peripheral blood B cells.

Fc receptors for IgM are more easily detected on normal T and B cells after overnight culture (Moretta *et al.*, 1975; Ferrarini *et al.*, 1977). In contrast, they are detected on freshly isolated CLL lymphocytes of many patients as shown in this and previous analyses (Pichler & Knapp, 1977; Ferrarini *et al.*, 1977). The reason for this and its significance is not fully understood. In order to determine whether IgE Fc receptor bearing cells could also be more easily detected after overnight culturing, normal lymphocytes (Hellström, Perlmann & Spiegelberg, 1978) and some of the CLL lymphocytes were cultured employing the method of Moretta *et al.* (1975). However, no increase or a slight decrease of $E_OA_E$ positive cells were found with both types of lymphocytes. No IgE antibodies are available to coat $E_O$ in a manner similar to the IgG and IgM sensitization. $E_O$ were therefore coated with IgE myeloma proteins either adsorbed onto aldehyde fixed $E_O'$ (Gonzalez-Molina & Spiegelberg, 1977), or the Fc fragment of IgE was bound to $E_O$ through an Fab′ anti-$E_O$ bridge. Both types of indicator cells gave similar percentages of positive cells. The fresh cells, however, did not have the same tendency to form spontaneous rosettes as did the $E_O'$, particularly on HCL cells, and the Fab′-Fc IgE complexes allowed the preparation of fresh $E_OA_E$ indicator cells within a relatively short time period as compared to $E_O'$ coated cells. The average of 1·8% of $E_OA_E$ positive cells in normal controls was somewhat lower than the 4% reported previously (Gonzalez-Molina & Spiegelberg, 1977). More normal donors need to be analysed in order to obtain a significant value for the $E_OA_E^+$ cells of the normal population. Salsano *et al.* (1976) used human red cells coated with human IgG (Ripley) antibodies and found most CLL lymphocytes to be negative for IgG Fc receptors. In contrast, Ferrarini *et al.* (1975) used $E_O$ sensitized with rabbit IgG antibodies and found the cells of most patients to be positive. In a more recent study (Ferrarini *et al.*, 1977), all patients with CLL, like those in our study, were positive for Fc IgG receptors.

It was not possible in this study to see whether treatment affected the numbers of cells expressing Fc receptors, since all but two patients had chemotherapy before being tested. Previously, it was reported that treatment of CLL patients causes an increase of marker-negative cells, but has little effect on the complement and Fc receptor characteristics of the remaining positive cells (Salsano *et al.*, 1976). One of our patients who was Fc IgE receptor negative became positive when tested 6 and 8 months later in a phase of progressive disease. It is possible, therefore, that the duration and course of the disease with or without the influence of treatment can result in a change of expression of Fc receptors in individual patients. Large numbers of Fc receptor positive cells were found only in B cell leukaemias; however, the numbers of patients with ALL or Sézary syndrome tested were too small to conclude that these receptors are found exclusively on leukaemic lymphocytes of CLL, LSL and HCL patients.

## ADDENDUM

Since we submitted this manuscript, thirteen additional patients with leukaemias have been tested. Four out of nine CLL patients had significant numbers of Fc IgE receptor positive cells (11%, 19%, 23 and 71%), whereas one patient with LSL, two with ALL and one with undifferentiated null cell leukaemia had less than 6%. In total, eight out of thirty or 26% of the patients with B cell leukaemias had significant numbers of Fc IgE receptor positive cells.

The authors thank Drs R. McIntire, K. Ishizaka and E. Eyster for plasma of IgE myeloma patients Sha and For, and Drs T. Lutz, H. Long, A. Melaragno, G. Luiken, J. Morrison, M. Medina, J. Worthman and D. Masys for allowing us to study the cells of their patients. The excellent technical assistance of Ms Gloria Portillo and Mrs Beverly Peltzer, the editorial assistance of Mrs Phyllis Minick and the preparation of the manuscript by Mrs Margaret Stone are very much appreciated.

This is publication no. 1459 from the Immunology Departments of the Research Institute of Scripps Clinic. This study was supported by grants from the Bureau of Medicine and Surgery, Clinical Investigation Program Project No. 7-16-1078 and NIH grant AI-10734-07.

## REFERENCES

BØYUM, A. (1968) Separation of leucocytes from blood and bone marrow. *Scand. J. clin. Lab. Invest.* **21**, Suppl. 97, 77.

CUATRECASAS, P., WILCHECK, M. & ANFINSEN, C.B. (1968) Selective enzyme purification by affinity chromatography. *Proc. nat. Acad. Sci. (Wash.)*, **61**, 636.

DICKLER, H.B. (1976) Lymphocyte receptors for immunoglobulin. *Adv. Immunol.* **24**, 167.

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

DICKLER, H.B., SIEGAL, F.P., BENTWICH, Z.H. & KUNKEL, H.G. (1973) Lymphocyte binding of aggregated IgG and surface Ig staining in chronic lymphocytic leukaemia. *Clin. exp. Immunol.* **14**, 97.

FERRARINI, M., TONDA, G.P., RISSO, A. & VIALE, G. (1975) Lymphocyte membrane receptors in human lymphoid leukaemias. *Europ. J. Immunol.* **5**, 89.

FERRARINI, M., HOFFMAN, T., FU, S.M., WINCHESTER, R. & KUNKEL, H.G. (1977) Receptors for IgM on certain human B lymphocytes. *J. Immunol.* **119**, 1525.

FRIDMAN, W.H., NEAUPORT-SAUTES, C., GUIMEZANES, A. & GISLER, R.H. (1975) Relations between Fc receptors of activated T cells, immunoglobulin binding factor (IBF) and suppression of *in vitro* antibody synthesis. *Membrane receptors of lymphocytes* (ed. by M. Seligmann, J. L. Preud'homme and F. M. Kourilsky), p. 217, North Holland, Amsterdam.

FRØLAND, S.S., WISLØFF, F. & MICHAELSEN, T.E. (1974) Human lymphocytes with receptors for IgG. A population of cells distinct from T- and B-lymphocytes. *Int. Arch. Allergy Appl. Immunol.* **47**, 124.

FU, S.M., WINCHESTER, R.J. & KUNKEL, H.G. (1974) Occurrence of surface IgM, IgD and free light chains on human lymphocytes. *J. exp. Med.* **139**, 451.

GMELIG-MEYLING, F., VAN DER HAM, M. & BALLIEUX, R.E. (1976) Binding of IgM by human T lymphocytes. *Scand. J. Immunol.* **5**, 487.

GONZALEZ-MOLINA, A. & SPIEGELBERG, H.L. (1976) Binding of IgE myeloma proteins to human cultured lymphoblastoid cells. *J. Immunol.* **117**, 1838.

GONZALEZ-MOLINA, A. & SPIEGELBERG, H.L. (1977) A subpopulation of normal human peripheral B lymphocytes that bind IgE. *J. clin. Invest.* **59**, 616.

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

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.

HELLSTRÖM, U.B., PERLMANN, P. & SPIEGELBERG, H.L. (1978) Characterization of human lymphocytes with Fc receptors for IgE. *Fed. Proc.* **37**, 1361.

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

KERBEL, R.S. & DAVIES, A.J.S. (1974) The possible biological significance of Fc receptors on mammalian lymphocytes and tumour cells. *Cell*, **3**, 105.

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

KNAPP, W., SCHUIT, H.R.E., BOLHUIS, R.L.H. & HIJMANS, W. (1974) Surface immunoglobulins in chronic lymphatic leukaemia, macroglobulinaemia and myelomatosis. *Clin. exp. Immunol.* **16**, 541.

KUBO, R.T., GREY, H.M. & PIROFSKY, B. (1974) IgD, a major immunoglobulin on the surface of lymphocytes from patients with chronic lymphatic leukaemia. *J. Immunol.* **112**, 1952.

LAWRENCE, D.A., WEIGLE, W.O. & SPIEGELBERG, H.L. (1975) Immunoglobulins cytophilic for human lymphocytes, monocytes and neutrophils. *J. clin. Invest.* **55**, 368.

LEVY, R., WARNKE, R., DORFMAN, R.F. & HAIMOVICH, J. (1977) The monoclonality of human B-cell lymphomas. *J. exp. Med.* **145**, 1014.

LOBO, P.I., WESTERVELT, F.B. & HORWITZ, D.A. (1975) Identification of two populations of immunoglobulin-bearing lymphocytes in man. *J. Immunol.* **114**, 116.

LOBO, P.I. & HORWITZ, D.A. (1976) An appraisal of Fc receptors on human peripheral blood B and L lymphocytes. *J. Immunol.* **117**, 939.

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

MORETTA, L., WEBB, S.R., GROSSI, C.E., LYDYARD, P.M. & COOPER, M.D. (1977) Functional analysis of two human subpopulations: help and suppression of B cell responses by T cells bearing receptors for IgM (TM or IgG (TG). *J. exp. Med.* **146**, 184.

NEAUPORT-SAUTES, C. & FRIDMAN, W.H. (1977) Charac-

terization of suppressive immunoglobulin-binding factor (IBF). II. Purification and molecular weight determination of IBF produced by L-5178-Y θ-positive lymphoma. *J. Immunol.* **119**, 1269.

NISONOFF, A., WISSLER, F.C., LIPMAN, L.N. & WOERNLY, D.L. (1960) Separation of univalent fragments from the bivalent rabbit antibody molecule by reduction of disulfide bonds. *Arch. Biochem. Biophys.* **89**, 230.

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

PICHLER, W.J. & KNAPP, W. (1977) Receptors for IgM-coated erythrocytes on chronic lymphatic leukemia cells. *J. Immunol.* **118**, 1010.

PORTER, R.R. (1959) The hydrolysis of rabbit gamma globulin and antibodies with crystalline papain. *J. Biochem.* **73**, 119.

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., BROUET, J.C., CLAUVEL, J.P. & SELIGMANN, M. (1974) Surface IgD in immunoproliferative disorders. *Scand. J. Immunol.* **3**, 853.

ROWE, D.S., HUG, K., FORNI, L. & PERNIS, B. (1973) Immunoglobulin D as a lymphocyte receptor. *J. exp. Med.* **138**, 965.

SALSANO, F., FRØLAND, S.S., NATVIG, J.B. & MANDELLI, F. (1976) Chronic lymphatic leukemia: Studies on the effect of drug treatment on different lymphocytic subpopulations. *Scand. J. Immunol.* **5**, 1185.

STRAUSBACH, P., SULICA, A. & GIVOL, D. (1970) General method for the detection of cells producing antibodies against haptens and proteins. *Nature (Lond.)*, **227**, 68.

UNANUE, E.R., GREY, H.M., RABELLINO, E., CAMPBELL, P. & SCHMIDTKE, 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.