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# Fc receptors on human blood B lymphocytes

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SUMMARY

The frequency of Fc-receptor positive B lymphocytes in human blood was investigated. Under the conditions used heat-aggregated gammaglobulin binding and EA(ox)-rosette formation labelled the same lymphocyte populations. Using various techniques, double marking and cell separations the proportion of Fc-receptor positive cells within the surface Ig carrying population was estimated to be between 11·8 and 36·2%. The proportion of SIg carrying cells within the population forming EA-rosettes was between 11 and 26·4%. This represents extreme values due to known technical circumstances.

## INTRODUCTION

Recent interest in lymphocyte subpopulations has led to several reports about their relative frequency, Some subpopulations have been easy to define due to unequivocal demonstration of their markers. such as T and surface Ig-carrying B cells. Definition of the presence of Fc receptor-bearing cells has been somewhat controversial. Fc receptor-bearing lymphocytes judged by binding of aggregated gammaglobulin have been considered to be B lymphocytes (Dickler & Kunkel, 1972; Dickler, 1974). Brain & Marston (1973) reported a coincidence of 73% Fc receptor—estimated by EA-rosettes and surface Ig-carrying cells, while Kurnick & Grey (1975) found only 11% SIgG cells among EA-rosettes if the cells were tested after incubation at 37°C. By this procedure they meant to release passively attached surface Ig and avoid falsely high figures due to binding of IgG molecules via the Fc receptors (Lobo *et al.*, 1975; Horwitz & Lobo, 1975). Frøland, Wisløff & Michaelsen (1974) proposed that the Fc-positive cells represent a unique (non-B, non-T) lymphocyte population. A dissociation between surface Ig and Fc receptor was also found on human lymphoblastoid cell lines (Jondal & Klein, 1973).

Recently Samarut, Brochier & Revillard (1976) reported that less than half of the B cells formed EA-rosettes. Their B markers were EAC-rosette formation and reactivity with anti-B cell/monocyte antibody (BLMCA). Using the same rosette technique combined with SIg staining Sandilands *et al.* (1976) found a much higher correlation between B cells and EA rosettes. In their experiments about one-third of the EA rosettes were formed by SIg cells and the majority of B cells had Fc receptors. Since the presence of a substantial Fc positive non-B- non-T-cell population in human blood is well documented, we focused our attention to investigate B cells for the presence or absence of easily detectable Fc receptors.

## MATERIALS AND METHODS

*Lymphocyte separation.* Blood was drawn from healthy volunteers. Lymphocytes were separated on Ficoll–Isopaque (Böyum, 1968) at room temperature. Macrophages were removed by carbonyl iron treatment (30 min, 37°C) in the presence of 20% foetal calf serum (FCS) (Jondal, 1974). Filtration through nylon wool column was carried out according to the method of Julius, Simpson & Herzenberg (1973) with slight modifications (Cornain *et al.*, 1975). $5 \times 10^7$ lymphocytes in 2 ml were poured into 5-ml plastic syringes filled with 0·4 g washed nylon wool (Fenwal Laboratories, U.S.A.), and incubated for 60 min at 37°C. Non-adherent cells were eluted with 10 ml warm (37°C) RPMI 1640. Adherent cells were removed by gentle agitation of the wool with FCS at 37°C.

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EA-rosette forming cells (EA-RFC) were separated on Ficoll–Isopaque gradients. The cells both in the pellet (enriched for EA-RFC) and in the interface (depleted for EA-RFC) were collected. For estimation of the proportion of EA-rosettes the pellet was gently resuspended and inspected. Cells were stained for SIg after lysing the pellet by 0·83% ammonium chloride treatment in the cold.

*Lymphocyte markers.* Before studies for markers in order to eliminate absorbed immunoglobulins, lymphocytes were kept for 2 hr at 37°C (with 5% $CO_2$, 95% air), and thereafter washed again. EA rosettes were formed according to the method of Hallberg, Gurner & Coombs (1973), 7S rabbit anti-ox red blood cell (ORBC) antibody was used for sensitization of ORBC. $10^6$ lymphocytes were mixed with 0·5% sensitized erythrocytes, incubated for 15 min at 37°C, pelleted at 100 *g* for 8 min, and further incubated for 30 min at 0°C. The pellet was gently resuspended, rosettes were counted by scoring 200 cells in each sample. Lymphocytes binding three or more erythrocytes were considered rosettes.

Surface Ig (SIg) staining was performed at 4°C. Lymphocytes were incubated with 1:8 diluted FITC-labelled polyvalent goat anti-human Ig (Hyland, U.S.A.) for 45 min. After three washes the cells were inspected in a Leitz Orthoplan fluorescence microscope equipped with a vertical illuminator. 200 cells were counted in each sample. All determinations were done in triplicates.

Aggregated gammaglobulin binding: FITC-labelled heat-aggregated (63°C, 15 min) human gammaglobulin (HAG) was prepared from Kohn II fraction human gammaglobulin (Kabi, Sweden) according to the method of Dickler & Kunkel (1972). Before use the preparation was centrifuged at 1000 *g* for 10 min. The conjugate was used in 1:10 dilution, based on reactivity with known Fc-receptor positive cells (Daudi lymphoblastoid cell line). Staining was performed at room temperature for 30 min, thereafter the cells were washed three times with RPMI 1640. Examination in the microscope was performed as described above. Brightly fluorescent cells were easily distinguishable from negative ones.

*Double marking experiments.* EA-rosette formation was combined with either SIg staining or HAG binding. Lymphocytes were treated with either HAG or stained for SIg as described above, thereafter the cells were washed three times and resuspended in RPMI 1640 medium and rosette formation with sensitized ORBC was performed.

## RESULTS

The distribution of SIg and Fc-receptor carrying cells in the lymphocyte fractions is shown in Table 1. Fc-receptors determined by HAG or EA rosetting gave similar results. Passage through nylon wool separated SIg (B) cells from other lymphocytes. While almost all SIg cells were retained by the column, part of Fc-receptor positive cells were not. In the adherent fraction, though almost half (44·2%) of the cells were SIg positive, the Fc positive cells were not enriched. Thus detected both with EA-rosettes and HAG binding, Fc-receptor positive cells are not identical with SIg positive B cells. Assuming that all SIg-Fc positive cells are in this fraction (probably untrue) 31·4% or 36·2% (calculated on the basis of per cent of HAG-positive cells or EA-rosettes related to SIg positive cells) of the B-cell population were Fc positive.

When the population was depleted for EA-RFC by Ficoll gradient sedimentation 10·5% SIg positive cells were still present and among the recovered rosettes 19% were SIg positive. Thus, effective elimination of EA-rosettes (72% were found in the pellet, and 3·5% in the interface) did not deplete SIg positive cells. The percentage of SIg-Fc positive cells in this EA rosette-enriched fraction was estimated to be 26·4%. Since the proportion of EA-rosettes decreased with 66·7% while the SIg cells only 10·3%, this estimates the EA-rosetted SIg cells.

HAG pretreatment interfered with EA-rosetting. The degree of blocking in the various experiments

TABLE 1. Proportion of SIg and Fc-receptor positive cells in different lymphocyte fractions

| | | Nylon wool | | EA-RFC | |
|---|---|---|---|---|---|
| | Unfractionated | non-adherent | adherent | depleted | enriched |
| SIg | 11·7±1·8* | 1·6±0·5 | 44·2±5·5 | 10·5±0·6 | 19·0±3·2 |
| Fc (HAG) | 15·2±2·4 | 10·3±1·1 | 13·9±1·8 | 3·8±0·6 | n.d.† |
| Fc (EA-RFC) | 10·5±1·6 | 13·0±2·3 | 16·0±2·0 | 3·5±0·7 | 72·0±7·0 |

Mean±s.e., $n=12$.

* Per cent.

† Not done, because EA-rosette formation strongly inhibited HAG binding.

varied. A number of cells had attached erythrocytes, but with the criteria used for positivity (three or more erythrocytes) these were counted as negatives. In such double marking experiments almost all (93%) of rosettes were formed around HAG fluorescing cells (Table 2).

Surface Ig staining interfered with subsequent EA-rosette formation only slightly. 9·1% EA-rosettes were seen after SIg staining (instead of the expected 13·5%). 11% of the rosette forming cells carried SIg and 11·8% of the SIg cells bound rosettes.

TABLE 2. Double marking experiments. Coincidence of SIg and EA-rosettes or HAG binding and EA-rosettes on blood lymphocytes

| | EA-RFC (%) | | | | | |
|---|---|---|---|---|---|---|
| Expt. no. | Without pretreatment | After SIg staining | After HAG binding | EA-RFC/HAG binding* | EA-RFC/SIg* | SIg/EA-RFC† |
| 1 | 14 | 14 | 3·5 | 33/55 (100)‡ | 50/4 (8)‡ | 8‡ |
| 2 | 6 | 6 | 0·5 | 33/30 (90·9) | 30/6 (20) | 7 |
| 3 | 18 | 9 | 1·5 | 26/25 (96·1) | 50/3 (6) | 15 |
| 4 | 14 | 9 | 1 | 13/13 (100) | 100/12 (12) | 17 |
| 5 | 14 | 8 | 11 | 22/18 (81·8) | 100/11 (11) | 10 |
| 6 | 15 | 8·5 | 13·5 | 28/25 (89·3) | 100/9 (9) | 14 |
| Mean ± s.e. | 13·5 ± 1·6 | 9·1 ± 1·1 | 5·2 ± 2·3 | 93 ± 2·9† | 11·0 ± 2·0‡ | 11·8 ± 1·7 |

* Total rosettes/fluorescing rosettes.
† Fluoresceing cells/fluorescing rosettes.
‡ Per cent values.

## DISCUSSION

Our findings suggest that under the test conditions used the same lymphocyte population binds HAG and EA(ox)-rosettes, since the percentages of Fc-positive cells obtained by the two methods were almost identical in every lymphocyte fraction. In the double marking experiments pretreatment with HAG blocked EA-rosetting. Those rosettes which were formed were almost all HAG positive. Thus, our results are at variance with that of Frøland, Natvig & Michaelsen (1974) who considered the two methods to detect different populations. They used different rosette techniques (i.e. erythrocytes coated with Ripley type anti-CD-antiserum).

The proportion of SIg and Fc-positive cells was found to be similar in the unfractionated population. That Fc-positive cells are only partly overlapping with the SIg positive B cells has been proven by three independent approaches.

(1) While nylon wool effectively removed SIg cells, part of the Fc-positive lymphocytes passed this column. The nylon wool adherent fraction enriched for SIg cells did not have a similar enrichment of Fc-positive cells. Since a considerable amount of lymphocytes are entrapped in the nylon wool column without real adherence, some Fc-positive nonadherent non-B cells contaminate this fraction, consequently the proportion of Fc-positive B cells is lower than the recorded value. Accordingly, the real frequency for Fc-receptor positive B cells retained by the column must be lower than 31·4–3·62%.

(2) Ficoll sedimentation enriched effectively the EA-rosettes, while the interface still contained SIg cells. 26·4% of the pelleted rosettes were SIg positive. Since centrifugation on Ficoll may have disrupted some rosettes, the right values for Fc-positive cells in the pellet is probably higher. On the other hand, part of the SIg cells in the pellet sedimented without being EA-rosettes. Taking into account these possibilities the estimated value for SIg-Fc-receptor positive lymphocytes in this EA-RFC enriched fraction may be lower than 26·4%. The estimated 10·3% Fc-positive cells among B cells (though a rough estimate) must probably be the lower limit, since not all Fc-positive cells went to the pellet.

(3) Estimated directly by double marking, only 11% of the EA-RFC carried surface Ig and 11·8%

of SIg positive cells rosetted. The SIg staining, however, slightly interfered with EA rosetting. The real values for both categories must therefore be higher.

In conclusion, according to the estimations achieved by three different approaches the majority of B cells (SIg cells) (63·8–88·2%) lacks Fc-receptors. Each technique used, involved different sources of errors. The direct approach, i.e. double labelling, represents the lower limit (11·8% Fc-positive cells among B cells) as well as the elimination of SIg positive cells concomitantly with EA-rosette sedimentation (10·3%). The upper limit is based on the analysis of nylon wool adherent population, estimating 31·4 or 36·2%. Similarly, the calculated values for the proportion of B cells among Fc-positive cells (10·3 and 26·4%) represent the lower and upper limits respectively. These values give an overlapping of Fc and SIg markers of approximately 1–3% in the total lymphocyte population.

Previous experiments (Dickler & Kunkel, 1972; Brain & Marston, 1973; Brown & Greaves, 1974; Dickler, 1974; Sandilands *et al.*, 1976), attempting to find correlation between SIg and Fc-receptor on human lymphocytes, counted probably non-B Fc-positive cells due to absorbed IgG as SIg cells since they did not attempt to release it by incubation. For example Sandilands *et al.* (1976) recorded about 90% EA-rosetting cells in the SIg population in combined marker test, and the depletion of SIg cells with depletion of EA rosettes. Samarut, Brochier & Revillard (1976) described 15·2% EA-rosetting cells and 14·7% B cells (our values are only slightly lower) in the blood. Our results correspond well with theirs with regard to the effect of nylon wool treatment and EA-rosette sedimentation though in the latter step they obtained higher degree of B-cell depletion (31%). They calculated 41% B cells in the EA-RFC and 45% EA rosettes in the B-cell population. Thus the proportion of Fc-positive B cells was estimated to be lower than in previous publications but still higher than in ours.

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