BINDING OF SOLUBLE IMMUNE COMPLEXES TO
HUMANLYMPOBLASTOID CELLS

I. Characterization of Receptors for IgG Fc and Complement and
Description of the Binding Mechanism*

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Human bone marrow-derived (B type) lymphocytes have receptors for IgG Fc (1)
and the C3b and C3d fragments of C3 (2, 3). The contribution of each receptor to
the binding of soluble immune complexes which have interacted with comple-
ment has not yet been thoroughly investigated. The present studies were under-
taken to characterize the binding of IgG Fc and complement by various lympho-
blastoid cell lines in the hope that these cells might serve as in vitro detectors of
immune complexes. As a model for immune complexes we employed aggregated
human gamma globulin (AHG)† before and after interaction with human serum
as source of complement, and observed its binding via receptors for IgG Fc, C3b,
and C3d on the lymphoblastoid cell surface. Human lymphoblastoid cell lines
with B-cell characteristics were chosen for study since it was felt that they would
be a more convenient and homogeneous indicator system than any other source
of lymphoid cells. Nine such lymphoblastoid cell lines were characterized for
membrane-bound Ig (MBIg) and receptors for IgG Fc and complement. The Raji

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† Abbreviations used in this paper: AHG, aggregated human gamma globulin; BSA, bovine serum
albumin; C, complement; C3bina, C3b inactivator; DEAE, diethylmethyl-cellulose; EA, antibo-
dy-sensitized sheep erythrocytes; EAC, complement coated sensitized sheep erythrocytes;
EAC1423b**, EA coated with isolated human C1, C4, C2, C3; EAC1423d, EA coated with human
C1, C4, C2, C3 and then rendered immune adherence negative; EAC1-3 mo, EA coated with C1-C3
from mouse serum deficient in C5; EAC1-5 rab, EA coated with C1-C5 from rabbit serum deficient in
C6; FITC, fluorescein isothiocyanate; GVB, Veronal-buffered (5 mM) saline containing 1.0% gelatin,
1.5 × 10⁻⁴ M CaCl₂, 5 × 10⁻⁴ M MgCl₂; HSA, human serum albumin; MBIg, membrane-bound
immunoglobulin; MEM, minimum essential medium; NHS, normal human serum; PBS, phos-
phate-buffered saline; RBC, red blood cell.
cell line lacking MBIg but having receptors for IgG Fc, C3b, and C3d was selected for assaying the contribution of these receptors to the binding of AHG. This study revealed that soluble AHG containing complement binds to the cells only via the receptors for C3b and C3d.

Materials and Methods

Lymphoblastoid Cell Lines. Raji and Daudi cell lines were derived from patients with Burkitt's lymphoma (4, 5). 8866 cells were derived from the peripheral blood of a patient with acute myelocytic leukemia (6) and Wil2WT cells were cultured from the spleen of a patient with hereditary spherocytic anemia (7). The SCRF5001, SCRF5004, and SCRF5005 human cell lines were provided by Dr. B. Croker, Scripps Clinic. The SCRF5001 and SCRF5004 lines were derived from peripheral blood of two patients with systemic lupus erythematosus; the SCRF5005 line was derived from the peripheral blood of a patient with Sjögren's syndrome. The Sommer 8432 and Sommer 8402 cell lines were provided by Dr. G. E. Moore, Denver General Hospital, Denver, Colo. and were cultured from the peripheral blood of a patient with acute lymphocytic leukemia. Suspension cultures of these cell lines were propagated in Eagle's minimum essential medium (MEM; Autopow, Flow Laboratories, Inc., Rockville, Md.) supplemented with glutamine, nonessential amino acids, pyruvate, 10% fetal bovine serum, penicillin, streptomycin, and fungizone, as described by Lerner et al. (8). Cell viability was determined by trypan blue exclusion.

C3 and C3b. Human Serum was prepared from human serum according to the method described by Nilsson and Müller-Eberhard (9). The C3b fragment was prepared by enzymatic cleavage of C3 with trypsin (10) and was isolated by column chromatography and pevikon electrophoresis. Aggregated Human Gamma Globulin (AHG). Human IgG was prepared by fractionation of human gamma globulin II (Miles Laboratories, Inc., Kankakee, Ill.) on a DEAE-52 cellulose column. IgG (10 mg/ml) was conjugated with fluorescein isothiocyanate (FITC) as in (11) and was aggregated subsequently by heating at 63°C for 20 min. Soluble fluorescein-conjugated aggregated human gamma globulin (FITC-AHG), prepared after centrifugation of the aggregates at 1,500 g for 30 min., was used in a concentration of 2 mg/ml of phosphate-buffered saline (PBS), pH 8.0. Radioiodination. AHG was iodinated with 125I according to the method of McConahey and Dixon (12). Human IgG (2 mg/ml) was first aggregated by heating (63°C, 30 min), freed of large insoluble aggregates by centrifugation at 1,500 g for 30 min and then iodinated. [125I]AHG was dialyzed against PBS for 24 h and then centrifuged at 2,000 g for 30 min in order to remove any insoluble aggregates. The specific activity of the preparation used was 3 x 10^5 cpm/μg protein. Antisera. Antihuman C3 serum was prepared by immunizing rabbits with isolated C3 (10). When tested by double diffusion in agar, the antihuman C3 serum (2 mg/ml) used in this study reacted with C3 and the fragments C3b, C3c, and C3d. This serum gave a single precipitin line when tested against normal human serum (NHS) or isolated C3 on Ouchterlony analysis and immunoelectrophoresis. The antitoxine C3 serum (1 mg/ml) was prepared in rabbits as described (13). The goat antirabbit C3 (0.8 mg/ml, lot 57411) and goat antiguinea pig C3 (1.2 mg/ml, lot 5632) sera were purchased from Cappel Laboratories, Inc. (Dowington, Pa.) and gave only one line when tested against the corresponding serum by Ouchterlony analysis and immunoelectrophoresis. The rabbit antihuman immunoglobulin serum (IgG + IgA + IgM; 1.5 mg/ml, lot 6684) was purchased from Cappel Laboratories. The rabbit antihuman IgG serum was prepared by immunizing rabbits with human IgG. The IgG fraction of all antisera listed above was used. Immunofluorescent Staining. Antisera were conjugated with FITC according to the method of Clark and Shepard (11). In a typical experiment, 5 x 10^9 cells in 50μl of MEM without Ca++, with 100 μg of isolated human C3 or C3b or 50 μl of human, mouse, rabbit or guinea pig serum (freshly obtained or previously stored at -70°C) were incubated with gentle agitation at 37°C for 30 min. Subsequently, cells were washed three times with MEM and incubated with gentle agitation for 30 min at 4°C with 25 μl of the corresponding FITC antisera to C3. The specificity of the staining was demonstrated by repeatedly absorbing FITC antihuman C3, FITC antirabbit C3 and FITC antitoxine C3 serum with EAC1423b (EAC1423b), EAC1-5 rabbit (EAC1-5 rab), or EAC1-3 mouse (EAC1-3 mo), respectively, and finding that the absorbed antisera did not stain cells reacted
with human, rabbit, or mouse C3. To demonstrate membrane-bound Ig (MBIg) on human lymphoid cells, a direct fluorescence technique was employed in which 50 μl of lymphoid cell suspension (5 × 10⁸ cells) were incubated (4°C, 30 min) with 25 μl of FITC antihuman Ig serum. To demonstrate binding of AHG to human lymphoblastoid cells, both direct and indirect techniques were employed. In the direct method, 5 × 10⁸ cells in 50 μl MEM were incubated (37°C, 30 min) with 25 μl of FITC AHG. In the indirect method, cells were first incubated (37°C, 30 min) with FITC AHG, washed three times with MEM, and then reacted (4°C, 30 min), so as to cross-link FITC AHG molecules possibly bound to cells, with nonconjugated rabbit antihuman IgG serum. Before use the FITC AHG was centrifuged at 1,500 g for 20 min in order to remove large aggregates. In the final step, cells were washed three times with MEM and resuspended in 10 μl of 10% bovine serum albumin (BSA) in PBS. Cell smears were air dried, fixed in 95% cold ethanol, and mounted under glycerogel. Wet preparations were made by sealing a drop of the cell suspension with paraffin under a cover slip. Smears were observed by using a Zeiss interference filter system utilizing transmitted light from a HBO 200 watt mercury light source. Since all cells in a given preparation stained to the same degree, we could semiquantitatively classify the intensity of stain from negative to 3+ for both surface immunoglobulin and aggregate binding.

Erythrocyte-Antibody Complement Complexes. EAC1423b hu cells were prepared from sheep erythrocytes (E) (1 × 10⁹ cells/ml) sensitized with rabbit antierythrocyte antibody (A), and incubated with purified human complement (C) components as previously described (14). EAC1423b hu were stored at a concentration of 1 × 10⁹ cells/ml GVB (Veronal-buffered saline containing 1.0% gelatin, 1.5 × 10⁻⁴ M CaCl₂ and 5 × 10⁻⁴ M MgCl₂). EAC1-3 mo and EAC1-5 rab cells were prepared by incubating (37°C, 30 min) sensitized sheep erythrocytes (5 × 10⁸ cells) in 1 ml mouse serum diluted 1:5 with GVB or rabbit serum diluted 1:2 with GVB. Cells were washed three times and stored in GVB (1 × 10⁹ cells/ml). The mouse serum was obtained from B10D2 old line mice (Jackson Laboratories, Bar Harbor, Maine) genetically deficient in C5 (15). The rabbit serum was obtained from C6-deficient rabbits (16). Each batch of EAC’ cells could be used over a period of 7 days.

EAC1423d hu cells were obtained by incubating (37°C, 120 min) 5 × 10⁹ EAC 1423b hu with a sufficient amount of C3b inactivator (C3bina) or heat-inactivated (56°C, 30 min) human serum to make them immune adherence negative. C3bina cleaves C3b into C3c and C3d and treatment of EAC1423b hu with C3bina results in release of C3c and retention of C3d on the red cell surface (17). EAC1423d hu are immune adherence negative.

Rosette Formation of Cells Bearing Receptors for Complement with EAC’. 2 × 10⁸ lymphoblastoid cells were incubated in small plastic Eppendorf conical tubes (Brinkman Instrument Co., Los Angeles, Calif.) with 5 × 10⁸ EAC’ in a total vol of 200 μl of MEM without Ca ++, at 37°C for 30 min, during which time the mixture was shaken twice by hand. Thereafter, the cells were suspended in 1 ml MEM and placed on ice for 5 min. Rosetted (four or more bound erythrocytes) and unrosetted lymphocytes were counted in a hematocytometer, using a Zeiss phase-contrast microscope, and the percent of rosette-forming cells was calculated.

Rosette Formation with Sheep Erythrocytes. The method of Jondal et al. (18), with the modification of Bentwich et al. (19) was employed. In preliminary experiments it was found that approximately 60% of human peripheral lymphocytes (average of 15 experiments) formed rosettes. None of the human lymphoblastoid cell lines studied here formed spontaneous rosettes with sheep erythrocytes.

Immune Adherence. Immune adherence experiments were performed in microtiter plates (Cooke Engineering Co., Alexandria, Va.). In a typical experiment, each well was filled with 25 μl of Veronal buffer, pH 7.5, T/20.1, containing 0.1% human serum albumin (HSA). 25 μl of 1 × 10⁸ EAC/ml were placed in the first well and dilutions were made with a 25 μl microtiter loop. After addition of 25 μl of human O group red cell suspension containing 2 × 10⁹ red cells per ml to all wells, the plates were agitated for 5 min and then incubated at 37°C for 30 min, after which time the sedimentation pattern was evaluated.

Trypsin Treatment. 5 × 10⁸ cells were incubated with 0.01% trypsin in MEM (wt/vol) for 15 min at 37°C and then washed twice with 0.1% soy bean trypsin inhibitor in MEM.

* C3b inactivator was a gift from Dr. N. R. Cooper, Scripps Clinic and Research Foundation.
Results

Membrane-Bound Ig and Receptors for IgG Fc. Six out of the nine human cell lines were found to carry MB Ig, as shown with a rabbit antihuman Ig serum (Table I). Daudi cells gave the most intense staining; 8866 and Wil 2WT cells were weakly positive. An intermediate intensity of staining was seen with SCRF5001, SCRF5004, and SCRF5005 cells. In contrast to the other cell lines, Raji, Sommer 8432 and Sommer 8402 cells were negative for MB Ig by immunofluorescence. The presence of receptors for IgG Fc on the different cell lines was shown by a direct method in which FITC AHG was used and a two-step indirect method in which cells preincubated with FITC AHG were reacted with nonconjugated antihuman IgG. As judged by intensity of fluorescence, the

| Cell type   | MB Ig Fluorescence | Intensity of Fluorescence | Receptors for IgG Fc* Fluorescence | Intensity of Fluorescence |
|-------------|--------------------|----------------------------|-----------------------------------|---------------------------|
|             | positive cells     |                            | positive cells                    |                           |
| Daudi       | 100                | 3+                         | 100                               | 2+                        |
| 8866        | 100                | 1+                         | 0                                 | Neg                       |
| Wil2WT      | 100                | 1+                         | 0                                 | Neg                       |
| SCRF5001    | 100                | 2+                         | 100                               | 1+                        |
| SCRF5004    | 100                | 2+                         | 100                               | 1+                        |
| SCRF5005    | 100                | 2+                         | 100                               | 1+                        |
| Raji        | 0                  | Neg                        | 0                                 | 1+                        |
| Sommer 8432 | 0                  | Neg                        | 100                               | 1+                        |
| Sommer 8402 | 0                  | Neg                        | 0                                 | Neg                       |

* Results given were obtained by the indirect method.

indirect method was more sensitive in detecting membrane bound AHG than the direct one. 8866, Wil2WT, and Sommer 8402 cells did not bind AHG with either method. Raji and Daudi cells were positive by both methods, whereas the SCRF5001, SCRF5004, SCRF5005, and Sommer 8432 cells were positive only by the more sensitive indirect method. The strongest intensity of staining was found with Daudi cells, which indicated the presence of a large number of receptors for Fc on this cell type. When the presence of MB Ig and receptors for IgGFc are compared, as shown in Table I, four categories of cell lines can be distinguished: (a) Daudi, SCRF5001, SCRF5004, and SCRF5005 cells which have both MB Ig and receptors for IgG Fc, (b) 8866 and Wil2WT cells which have MB Ig but lack receptors for IgG Fc, (c) Raji and Sommer 8432 cells which have no MB Ig but which have receptors for IgG Fc, and (d) Sommer 8402 cells which have neither MB Ig nor receptors for IgG Fc.

Receptors for Erythrocyte-Bound C3b and C3d. Receptors for red cell-bound C3b and C3d have been found on human B-type lymphocytes (3, 20) and on Raji cells (14). In the present studies we tested the different cell lines for the presence
of these receptors by rosette formation with EAC1423bhu, EAC1423dhu, and EAC1-3 mo. EAC1-3 mo prepared with C5-deficient mouse serum have been shown to carry C3d (3). These cells gave no immune adherence reaction with human group O erythrocytes. Table II shows the percent of cells of each cell line forming rosettes with sensitized cells carrying C3b or C3d. Three categories of cells can be distinguished: (a) Raji, SCRF5001, SCRF5004, SCRF5005, and Sommer 8432 cells which have receptors for red cell-bound C3b and C3d, (b) Daudi and Sommer 8402 cells which have receptors only for red cell-bound C3d, and (c) 8866 and Wil2WT cells which lack both receptors.

**Distinction Between the Receptors for C3-C3b and C3d.** It has been shown that the receptor for C3 and C3b is the same, because C3, C3b, or NHS inhibited Raji-EAC1423bhu rosette formation (14). Since certain cells formed rosettes with both EAC1423b and EAC1423d, the possibility of the receptors for C3b and C3d being identical had to be investigated. In order to answer this question inhibition of rosette formation between Raji cells and either C3b or C3d carrying sensitized red cells with isolated human C3 or C3b and human and mouse serum containing C3 was attempted. Table III shows that preincubation of Raji cells with isolated human C3 or C3b, as well as with human or mouse serum, inhibited rosette formation with EAC1423bhu but did not have any effect on rosette formation with EAC1423dhu and EAC1-3 mo. These results indicate that the receptor for C3-C3b and the receptor for C3d are different.

**Table II**

| Cell type         | Percentage of lymphoid cells forming rosettes with: |
|-------------------|------------------------------------------------------|
|                   | EAC1423bhu | EAC1423dhu | EAC1-3 mo |
| Daudi             | 0          | 35         | 42        |
| 8866              | 5          | 2          | 2         |
| Wil2WT            | 2-3        | 1          | 0         |
| SCRF5001          | 100        | 89         | 85        |
| SCRF5004          | 100        | 92         | 90        |
| SCRF5005          | 100        | 90         | 90        |
| Raji              | 100        | 92         | 100       |
| Sommer 8432       | 100        | 87         | 95        |
| Sommer 8402       | 0          | 53         | 60        |

**Binding of Soluble C3 or C3b and RBC Bound C3b to Lymphocyte Receptors for C3-C3b.** We have previously demonstrated that soluble C3, soluble C3b, and RBC-bound C3b bind to the same receptor (14). Therefore, receptors for C3-C3b on lymphoid cells can be shown by staining cell bound C3 or C3b with FITC anti-C3 serum or by rosette formation with EAC1423bhu. The requirements for rosette formation and binding of soluble C3 and C3b may be different, resulting in different sensitivities of the two methods. Since certain cell lines did not form rosettes with EAC1423b, we investigated whether these cells were able...
to bind soluble C3 or C3b. Thus, the ability of the nine human cell lines to bind soluble C3 and C3b was compared to their ability to form rosettes with C3b carrying sensitized red cells, i.e., EAC1423b<sub>hu</sub> and EAC1-5 rab. EAC1-5 rab, although prepared with C6-deficient rabbit serum, carry C3b as demonstrated by positive immune adherence reactions with human group O erythrocytes. The results summarized in Table IV indicate that with most cell lines (Raji,

**TABLE III**

*Effect on Rosette Formation by Treatment of Raji Cells with Human C3 or C3b and Human or Mouse Serum*

| Treatment* | Indicator cells | Rosettes  |
|------------|-----------------|-----------|
| Raji + C3  | EAC1423b<sub>hu</sub> | 0         |
|            | EAC1423d<sub>hu</sub> | 90        |
|            | EAC1-3 mo       | 85        |
| Raji + C3b | EAC1423b<sub>hu</sub> | 0         |
|            | EAC1423d<sub>hu</sub> | 92        |
|            | EAC1-3 mo       | 87        |
| Raji + human serum | EAC1423b<sub>hu</sub> | 0         |
|            | EAC1423d<sub>hu</sub> | 89        |
|            | EAC1-3 mo       | 87        |
| Raji + mouse serum | EAC1423b<sub>hu</sub> | 0         |
|            | EAC1423d<sub>hu</sub> | 90        |
|            | EAC1-3 mo       | 83        |

* 2 x 10^6 Raji cells were incubated with 150 μg human C3 or C3b and 100 μl of fresh human or mouse serum, washed three times, and then incubated with the different indicator EAC'.

**TABLE IV**

*Comparison of the Binding of Soluble C3 or C3b with the Binding of RBC Bound C3b to Lymphocyte Membrane C3-C3b Receptors*

| Cell type   | Percentage of lymphoid cells forming rosettes with: | Percentage of lymphoid cells binding |
|-------------|-----------------------------------------------------|--------------------------------------|
|             | EAC1423b<sub>hu</sub>  | EAC1-5 rab  | Human C3 or C3b* | Rabbit C3‡ |
| Daudi       | 0                                   | 2         | 90                  | 100         |
| 8866        | 5                                   | 3         | 5                   | 83          |
| Wil2WT      | 2-3                                 | 1         | 2-3                 | 65          |
| SCRF5001    | 100                                 | 80        | 100                 | 100         |
| SCRF5004    | 100                                 | 70        | 100                 | 92          |
| SCRF5005    | 100                                 | 85        | 100                 | 90          |
| Raji        | 100                                 | 100       | 100                 | 100         |
| Sommer 8432 | 100                                 | 100       | 100                 | 100         |
| Sommer 8402 | 0                                   | 0         | 0                   | 0           |

* Cells were incubated with 100 μg human C3 or C3b and stained with FITC rabbit antihuman C3.

‡ Cells were incubated with 50 μl rabbit serum and stained with FITC goat antirabbit C3.
SCRF5001, SCRF5004, SCRF5005, Sommer 8402) there was good correlation between their ability to bind soluble C3 and C3b and their ability to form rosettes with EAC1423bhu and EAC1-5 rab. However, with certain cell lines (Daudi, 8866, Wil2WT) the detection of receptors for C3-C3b by immunofluorescence was more sensitive than by the rosette technique. For example, Daudi cells bound soluble human C3 and C3b as well as rabbit C3 but were unable to form rosettes with EAC1423bhu or EAC1-5 rab; 8866 and Wil2WT cells bound soluble C3 from rabbit serum but did not form rosettes with EAC1-5 rab.

The discrepancy between the results obtained for binding soluble C3-C3b and for binding red cell-bound C3b to certain cells may be explained by a different distribution of receptors on the cell membrane. In effect, distribution of the C3-C3b receptors on Raji and other cell lines able to form rosettes with C3b carrying red cells different than the distribution on Daudi cells unable to form rosettes with C3b carrying red cells was observed by immunofluorescence. As shown in Fig. 1, Raji cells carrying C3 or C3b and stained with FITC antihuman C3 showed a uniform, fine granular staining pattern whereas Daudi cells carrying C3 or C3b showed a reticulated distribution of the stain on the cell surface.

Species Specificity of the C3-C3b Receptor on Human Lymphoblastoid Cells. In the preceding paragraph it was shown that 8866 and Wil2WT cells bound rabbit C3 but not human C3 whereas other cell lines bound C3 of both species, which indicated differences in specificity of C3-C3b receptors. In the following experiment, the species specificity of the C3-C3b receptor on human lymphoblastoid cells was analyzed by using C3 of different species. Cells were incubated with isolated human C3 and C3b as well as human, mouse, rabbit, and guinea pig sera as sources of C3 of the different species. Cells were stained with the corresponding fluoresceinated anti-C3 serum. The results summarized in

Fig. 1. Demonstration of different immunofluorescent staining patterns of C3 or C3b bearing Raji and Daudi cells when stained with FITC anti-C3 serum. (a) Raji cells with uniform, fine granular staining. (b) Daudi cells with reticulated distribution of the stain. × 1,234.
Table V indicate a broad spectrum of specificities of C3-C3b receptors on some of the cells. Raji, Daudi, SCRF5001, SCRF5004, SCRF5005, and Sommer 8432 cells bound C3 of human, mouse, rabbit, and guinea pig origin, as well as isolated human C3 and C3b. In contrast, 8866 and Wil2WT cells bound virtually no human C3 and C3b but interacted with mouse, rabbit, and guinea pig C3. Finally, Sommer 8402 cells did not bind isolated human C3 and C3b nor C3 of any of the other species. Of all cell types tested with human, mouse, rabbit, and guinea pig C3, Raji and Daudi cells showed the strongest fluorescent staining which indicated that these cells had a large number of C3-C3b receptors. The

| Cell type       | Cells binding human | Cells binding C3 present in sera of: |
|-----------------|---------------------|-------------------------------------|
|                 |                     | C3  | C3b  | Human | Mouse | Rabbit | Guinea pig |
| Daudi           | 90                  | 85  | 90   | 100   | 100   | 85     |
| 8866            | 5                   | 5   | 5    | 80    | 83    | 90     |
| Wil2WT          | 2-3                 | 2   | 2-3  | 70    | 65    | 70     |
| SCRF5001        | 100                 | 100 | 100  | 95    | 100   | 100    |
| SCRF5004        | 100                 | 100 | 100  | 92    | 90    |
| SCRF5005        | 100                 | 100 | 100  | 90    | 100   |
| Raji            | 100                 | 100 | 100  | 100   | 95    |
| Sommer 8432     | 100                 | 100 | 100  | 100   | 70    |
| Sommer 8402     | 0                   | 0   | 0    | 0     |

* Cells were incubated with 100 μg human C3 or C3b and stained with FITC rabbit antihuman C3.

† Cells were incubated with 50 μl of the different sera and stained with the corresponding FITC anti-C3 serum.

results showed that C3-C3b receptors on the lymphoblastoid cells tested have different species specificities.

Identification of Receptors for IgG Fc and Receptors for C3-C3b and C3d. Since AHG as well as C3, C3b, and C3d all bound to certain cell types, we questioned whether receptor sites for IgG Fc were identical to either of the two receptors for complement, i.e., the receptors for C3-C3b or C3d. That the receptors for IgG Fc and the receptors for complement are different was shown by inhibition experiments. As shown in Table VI, preincubation of 5 × 10⁸ Raji cells with 100 μg AHG did not inhibit binding of C3 or C3b and preincubation with 100 μg C3 or C3b did not inhibit binding of AHG to the cells. Furthermore, preincubation of cells with AHG did not interfere with rosette formation with EAC 1423b hu and EAC1423d hu.

Contribution of Receptors for Complement and IgG Fc to Binding of AHG
Table VI

Demonstration of Distinct Receptors for IgG Fc and C3-C3b on Raji Cells

| 1st incubation | 2nd incubation | 3rd incubation | Fluorescence positive cells |
|----------------|----------------|----------------|-----------------------------|
| Raji + AHG C3  | FITC Rabbit antihuman C3 | 100 |
| Raji + AHG C3b | FITC Rabbit antihuman C3b | 100 |
| Raji + AHG NHS | FITC Rabbit antihuman C3 | 100 |
| Raji + C3 FITC AHG | Rabbit antihuman IgG | 100 |
| Raji + C3b FITC AHG | Rabbit antihuman IgG | 100 |

Containing Complement to Cells. After describing the receptors for IgG Fc, C3-C3b, and C3d on the various human lymphoblastoid cells, we employed the characterized cells to study each receptor’s contribution to the binding of immune complexes to these cells. Immune complexes in fresh serum may bind to cells via either the receptors for complement or via the receptors for IgG Fc, or both. Since whole serum contains C3b inactivator which can cleave C3b into C3c and C3d, immune complexes in whole serum may contain C3b, or C3d, or both C3 fragments. AHG was employed as an immune complex because it has been shown that AHG possesses many of the properties of antigen-antibody complexes (21, 22). In order to find out which of the receptors (IgG Fc, C3-C3b, C3d) mediates binding of complexes which have interacted with serum as the source of complement, the following experiments were performed. Different batches of 2.5 × 10⁴ Raji cells were incubated (37°C, 30 min) with 200 μg human IgG (cells with blocked receptors for IgG Fc), washed twice with medium, and then reacted (37°C, 45 min) with 5 μg of [¹²⁵I]AHG in 20 μl MEM, in 20 μl heated (56°C, 30 min) human serum or in 20 μl fresh human serum. Cells which had not been preincubated with IgG (cells with nonblocked receptors for IgG Fc) were also reacted with 5 μg [¹²⁵I]AHG in 20 μl MEM, in 20 μl heated human serum or in 20 μl fresh human serum. As depicted in Fig. 2, there was virtually no uptake by cells with blocked receptors for IgG Fc of [¹²⁵I]AHG in MEM or in heated serum, whereas cells with nonblocked receptors for IgG Fc bound between 0.1 and 0.15 μg of these aggregates. However, there was virtually no difference between the two types of cells in uptake of aggregates containing complement. The results indicate that AHG containing complement binds to cells only via the receptors for complement, since blocking of the receptors for Fc did not have any effect on the binding of these aggregates. It should be noted (Fig. 2) that the uptake of AHG containing complement was approximately eight times higher than that of AHG without complement. The fact the AHG containing complement bound to Raji cells only via the receptors for complement was also demonstrated in experiments in which the receptors for complement were removed from the cells by trypsin treatment. We showed previously that trypsin destroys the receptors for C3-C3b on Raji cells (14). In the present study it was found that trypsin also destroys the receptors for C3d on Raji cells since trypsin-treated Raji cells were unable to form rosettes with EAC1423d₉₀ or EAC1-3 mo. However, the receptors
for IgG Fc on Raji cells were resistant to trypsin since treated cells were still able to bind AHG. Utilizing trypsin-treated and untreated cells we were able to assess the contribution of the receptors for IgG Fc and complement to the binding of immune complexes to cells. As shown in Table VII, FITC AHG incubated with fresh human serum as the source of complement, only bound to nontrypsin-treated cells whereas FITC AHG or FITC AHG incubated with heat-inactivated

![Graph showing uptake of [125I]AHG by Raji cells in the presence or absence of complement.](image)

**Fig. 2.** Uptake of [125I]AHG by Raji cells in the presence or absence of complement. Aliquots of 2.5 × 10⁶ Raji cells with nonblocked and blocked receptors for IgG Fc were incubated with 5 μg of [125I]AHG in MEM, in fresh human serum or in heat-inactivated (56°C, 30 min) human serum. The IgG Fc receptors were blocked by incubating the cells with human IgG.

**Table VII**

**Demonstration of the Binding of AHG Containing Complement to Raji Cells via the Complement Receptors**

| Cells  | Treatment* | Incubation† | Positive cells |
|--------|------------|-------------|----------------|
| Raji   | None       | FITC AHG    | 100            |
| Raji   | Trypsin    | FITC AHG    | 100            |
| Raji   | None       | FITC AHG + heated HS | 100         |
| Raji   | Trypsin    | FITC AHG + heated HS | 100         |
| Raji   | None       | FITC AHG + NHS | 100          |
| Raji   | Trypsin    | FITC AHG + NHS | 3.5          |

*5 × 10⁶ Raji cells were incubated (37°C, 15 min) with MEM or with a 0.01% (wt/vol) solution of trypsin in MEM. The effect of trypsin was stopped by washing the cells three times with 0.1% solution of soy bean trypsin inhibitor in MEM.

†Trypsin and nontrypsin treated cells were incubated (37°C, 30 min) with FITC AHG in medium or with FITC AHG which had reacted (37°C, 30 min) with heat-inactivated (56°C, 30 min) or fresh NHS.
(56°C, 30 min) human serum bound to both nontryptsin- and trypsin-treated Raji cells. The results again indicate that binding of AHG which had interacted with complement is solely mediated by the receptors for complement.

**Contribution of the Receptors for C3-C3b and C3d to the Binding of AHG-Containing Complement to Cells.** In the preceding section we demonstrated that AHG in fresh serum binds to cells only via the receptors for complement. However, since Raji cells have both receptors for C3-C3b and C3d and immune complexes incubated with whole serum may contain C3b, C3d, or both C3 fragments, experiments were carried out in order to define which of the two receptors for complement is operative in the binding of immune complexes to the cells. In these experiments 125I-labeled AHG and FITC AHG were employed. Different batches of 2.5 × 10⁶ Raji cells were incubated with 100 µg C3, 100 µg C3b, 50 µl NHS, or 50 µl MEM. The washed cells were then reacted with 5 µg [125I]AHG containing complement and the uptake of [125I]AHG by the different batches of cells was measured. Cells preincubated with C3, C3b, or NHS bound only approximately 50% of the amount of [125I]AHG taken up by the cells incubated in MEM (Fig. 3). Similar results were obtained when FITC AHG-containing complement was used; these aggregates also bound to cells with blocked receptors for C3-C3b. Thus, when cells were incubated with isolated human C3, C3b, or human serum and then reacted with FITC AHG which had previously interacted with whole human serum, fluorescence staining of the cells was observed. The results showed that AHG-containing complement binds to cells via both receptors for C3-C3b and C3d.

**Release of AHG-Containing Complement from the Cell Surface.** It has been previously shown by Miller et al. (23) that immune complexes containing complement bound to mouse lymphocytes can be released from the cell surface.
by incubating the cells in fresh whole human or mouse serum, but not by incubating them with isolated human C3 or C3b. The mechanism of this phenomenon has not been fully elucidated. Therefore, we decided to investigate the release phenomenon by using Raji cells. Different batches of $2.5 \times 10^6$ Raji cells coated with a certain amount of $[^{125}\text{I}]$AHG containing complement were prepared by incubating each batch of cells with 5 $\mu$g of these aggregates. Subsequently, AHG-coated cells were incubated (37°C, 45 min) with different amounts of human serum, purified human C3 or C3b. Control AHG-coated cells were incubated with medium containing 0.1% HSA. After incubation, supernates were collected and radioactivity present in supernates and on the cells was assessed. As depicted in Fig. 4, approximately 44% of the $[^{125}\text{I}]$AHG was released from the cells by 100 $\mu$l of whole fresh NHS containing 150 $\mu$g C3. As is shown also in Fig. 4, when 100 $\mu$l of a solution containing 150 $\mu$g human C3 or C3b was added to $[^{125}\text{I}]$AHG-coated cells, approximately 41% and 34% of the bound AHG was released, respectively. The percent of released radioactivity was decreased proportionately with the amount of serum, C3 or C3b used. The results indicate that NHS and purified human C3 or C3b can release some AHG containing complement bound to Raji cells.

Discussion

In the present work, we studied MBig and receptors for IgG Fc and complement of nine different human lymphoblastoid cell lines having B-type cell characteristics. Some of the cell lines studied expressed all of the described B-type lymphocyte markers (MBig, receptors for IgG Fc, receptors for complement). However, other cell lines showed only some of these markers. For example, we found cell lines without MBig but with receptors for IgG Fc and complement, cell lines with MBig and receptors for complement but without receptors for IgG Fc,
and cell lines without MBlg and receptors for IgG Fc but with receptors for complement. The data suggest that cell lines lacking certain B-cell markers may be derived from B cells which did not express all the surface markers, such as B cells at different stages of maturation or B cells belonging to different subclasses. On the other hand, cultured cells may have lost surface receptors which were present on the cells from which they were derived. For example, it has been shown that three subclasses of B-type lymphocytes are present in the blood of patients with chronic lymphocytic leukemia; cells having only MBlg, cells having only receptors for C3 and cells having both MBlg and receptors for C3 (24). In addition, in patients with X-linked agammaglobulinemia, lymphocytes without MBlg but with receptors for C3 have been described (25). Furthermore, loss of MBlg has been reported to occur in cultured cells (26).

Dickler et al. (27) have shown with human peripheral lymphocytes that there is a correlation between presence of MBlg and receptors for IgG Fc. The cell lines studied here did not show such an absolute correlation. For example, as noted above, cell lines without MBlg but with receptors for IgG Fc (Raji, Sommer 8432) and cell lines with MBlg but without receptors for IgG Fc (Wil2WT, 8866) were encountered. Absence of receptors for IgG Fc on Wil2WT and 8866 cells was shown not only by the direct fluorescent method but also by the more sensitive indirect method, which, according to Dickler et al. (27) and our own experience, can detect binding to cells of small size aggregates or 7S IgG.

Two types of receptors for complement were found to be present on the cell lines tested, one for C3 and C3b and one for C3d. Using sensitized red cells carrying C3b or C3d, it was found that some cell lines have both receptors for C3b and C3d, while others have only receptors for C3d, and others have neither receptor. By using the rosette test, it was shown that all cell lines having receptors for C3b also had receptors for C3d. Similar results with other cell lines have been reported by Ross et al. (3) and Dierich et al. (28).

In addition to the rosette test, the more sensitive fluorescent technique was used for detecting C3-C3b receptors on cells. By employing this technique it was found that cell lines unable to form rosettes with EAC1423bhu and EAC1-5 rab bound soluble C3 and C3b. Sommer 8402 is the only cell line of those tested which did not reveal a C3-C3b receptor by either method. Since this cell line formed rosettes with EAC1423dhu and EAC1-3 mo, we concluded that it has only the C3d receptor.

The different sensitivities of the immunofluorescence and the rosette techniques in demonstrating C3-C3b receptors may be due to the fact that EAC' is a large particle and in order to attach to cells it may require a special arrangement as well as a large number of C3-C3b receptors on the surface of the lymphoid cells. In fact, a difference in the distribution of the receptors for C3-C3b was observed by immunofluorescence between cells able to form rosettes with EAC1423bhu and EAC1-5 rab, and cells (Daudi) which were unable to form rosettes with EAC1423bhu and EAC1-5 rab. That the rosette technique does not always detect C3-C3b receptors on the cell surface is also supported by the demonstration of Dierich (personal communication), who found C3 receptor activity in solubilized cell extracts from lymphoid cell types which do not form rosettes with EAC1423bhu. Moreover, Nelson and Uhlenbruck (29) found that
mucoids derived from bovine erythrocytes inhibited the interaction of EAC-1423b hu with human erythrocytes which carry C3b receptors, whereas no interaction was observed with bovine red cells by themselves and EAC1423b hu.

In several reports on the classification of peripheral human lymphocytes as T and B cells, it has been stated that the percentage of B cells having MBIg was higher than that of B cells having C3b receptors (24, 30, 31). In those studies, C3b receptors were demonstrated by the rosette technique. Since the fluorescence method is more sensitive in detecting C3b receptors than the rosette technique, it may reveal a percentage of C3b receptor-bearing cells similar to that of Ig-bearing cells. We had already found that approximately 20% of human peripheral lymphocytes bound soluble C3b (14).

We have previously shown that the receptors for C3 and C3b are identical (14). In the present study we demonstrated that the receptors for C3-C3b are distinct from the receptors for C3d. Other experimental data also support the concept that C3-C3b and C3d receptors are independent. Ross et al. have shown that human red cells have only C3b receptors (3). The same authors used antisera to C3b and C3d receptors to show that the two receptors are distinct on the surface of human peripheral lymphocytes and cells of lymphoid cell lines (3).

A broad spectrum of species specificity of C3-C3b receptors on the human lymphoblastoid cell lines studied was shown by using C3 of different species. Whereas the majority of cell lines bound human C3 and C3b as well as mouse, rabbit, and guinea pig C3, some cell lines did not bind human C3 and C3b but were able to bind mouse, rabbit and guinea pig C3. The reason that some cell lines did not bind human C3 and C3b but bound C3 from other species is not understood. It may be that the specificity of C3 receptors of human lymphoid cells is altered in culture.

The receptors for IgG Fc and the receptors for C3-C3b and C3d are distinct since inhibition experiments demonstrated that Raji cells with blocked receptors for IgG Fc were able to bind soluble C3 and C3b and to form rosettes with sensitized red cells carrying C3b or C3d. The possibility that the receptors for IgG Fc, C3-C3b, and C3d are a class of Ig is excluded by the fact that Raji cells do not carry MBIg.

The contribution of each of the three receptors to the binding of soluble immune complexes containing complement to cell surfaces was assessed by using Raji cells which have IgG Fc, C3-C3b, and C3d receptors. It was demonstrated that the receptors for complement are far more important in binding immune complexes containing complement than receptors for Fc. In fact, it was shown that the binding of AHG containing complement was primarily mediated via the receptors for complement, since the amount of AHG containing complement bound to cells with or without blocked receptors for Fc was virtually the same. In contrast, the binding of AHG without complement was almost completely inhibited when cells with blocked receptors for Fc were used. The importance of complement receptors in the binding of complexes containing complement to cells was further demonstrated by using Raji cells with and without receptors for complement. Receptors for complement, but not IgG Fc, were removed from the cells by mild trypsin treatment. When these cells were used, it was found that AHG without complement bound to Raji cells with and without complement
receptors, whereas AHG containing complement bound only to complement receptor carrying Raji cells. Our findings indicate that the increased binding to cells of AHG containing complement, as compared to that of AHG without complement, is not due to additive binding via both Fc and complement receptors but is due to binding via complement receptors only. These results are in agreement with the studies of Eden, Bianco, and Nussenzweig, who have postulated that the complement mediated binding affects and impedes the Fc mediated binding of immune complexes to lymphocytes (32). According to these authors, complement bound to the antibody within the complexes may sterically hinder or directly bind to the sites on the Ig molecules which have affinity for the lymphocyte membrane. Although soluble aggregates containing complement bind to cells primarily via complement receptors, it should be noted that in other systems it has been shown that receptors for IgG Fc play a significant role in other cell functions such as phagocytosis of EAC

In the course of complement activation, C3b binds to immune complexes. Complex-bound C3b is thereafter cleaved by the serum enzyme C3b inactivator into C3c and C3d, with the d portion remaining on the immune complex (17). Therefore, immune complexes in whole serum may contain C3b, C3d, or both. Experiments presented here indicated that both receptors for C3-C3b and C3d are operative in binding of immune complexes coated with complement to cells. These experiments also suggested that Raji cells have approximately the same number of receptors for C3-C3b and C3d on their surface since it was found that 50% of aggregates that bound to cells with nonblocked receptors for C3-C3b and C3d on their surface since it was found that 50% of aggregates that bound to cells with nonblocked receptors for C3-C3b could still bind to cells with blocked receptors for C3-C3b.

Miller, Saluk, and Nussenzweig showed that immune complexes containing complement bound to murine B lymphocytes could be released from the cells by addition of NHS but not by addition of isolated human C3 or C3b (23). The authors postulated that release is due to activation of the alternate pathway of the complement system by complex-coated cells, which results in the generation of products which modify or cover the C3 binding sites on the complexes which are then released. In our experiments, NHS as well as isolated soluble human C3 and C3b could release a substantial percentage of immune complexes bound to Raji cells via receptors for complement. Since in our study not only whole serum but also isolated C3 or C3b were able to release immune complexes bound to Raji cells, we postulated that release of complexes from Raji cells by human serum is due to the presence in serum of C3 or C3b. C3b could be generated by activation of complement by immune complexes at the site of binding. We have previously shown that C3 or C3b competes with the red cell-bound C3b (EAC 1423b) for receptors for C3-C3b on Raji cells because addition of C3 or C3b to Raji-EAC 1423b rosettes resulted in dissociation of rosettes (14). Additional evidence of competition between free C3 and C3b and complexes containing complement for the receptors for complement has been obtained by other experiments in which uptake of AHG by Raji cells was greatly diminished when an excess of human serum was present.3 NHS as well as C3 and C3b were unable to release more than approximately 50% of cell-bound complement-coated aggregates, which may

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again indicate that part of immune complexes are bound to cells via the receptors for C3d.

Summary

In the present work we studied the expression of membrane-bound Ig (MBIg) as well as receptors for IgG Fc and complement on nine human lymphoblastoid cell lines. When MBIg and receptors for IgG Fc were compared, four categories of cell lines could be distinguished: (a) cell lines having both MBIg and receptors for IgG Fc, (b) cell lines having MBIg but lacking receptors for IgG Fc, (c) cell lines lacking MBIg but having receptors for IgG Fc, and (d) cell lines lacking both MBIg and receptors for IgG Fc. Two types of receptors for complement could be detected on the cell lines studied, one for C3-C3b and one for C3d. When sensitized red cells carrying C3b or C3d were used for rosette tests, three categories of cell lines could be distinguished: (a) cell lines having receptors for C3b and C3d, (b) cell lines having receptors only for C3d and (c) cell lines lacking both receptors. However, when a more sensitive immunofluorescent method was used instead of the rosette technique, it was found that cell lines unable to form rosettes with EAC1423bhu were able to bind soluble C3 or C3b which indicated the presence of these receptors on the cell surface. Inhibition experiments showed that receptors for C3-C3b and receptors for C3d are distinct and that receptors for C3-C3b and C3d are different from receptors for IgG Fc.

A cell line (Raji) without MBIg but with receptors for IgG Fc, C3-C3b, and C3d was selected for use in studying the binding mechanism of soluble immune complexes to cell surface membrane. Aggregated human gamma globulin was used in place of immune complexes. Immune complexes containing complement bind to Raji cells only via receptors for complement, namely receptors for C3-C3b and C3d. Binding of immune complexes containing complement to cells is much greater than that of complexes without complement. Immune complexes bound to cells via receptors for complement can be partially released from the cell surface by addition of normal human serum as well as isolated human C3 or C3b. We postulate that such release is due to competition of immune complex bound C3b and free C3 or C3b for the receptors on Raji cells.

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References

1. Dickler, H. B., and H. G. Kunkel. 1972. Interaction of aggregated γ-globulin with B lymphocytes. J. Exp. Med. 136:191.
2. Lay, W. H., and V. Nussenzweig. 1968. Receptors for complement on leukocytes. J. Exp. Med. 128:991.
3. Ross, G. D., M. J. Polley, E. M. Rabellino, and H. M. Grey. 1973. Two different
complement receptors on human lymphocytes. One specific for C3b and one specific for C3b inactivator-cleaved C3b. J. Exp. Med. 138:798.
4. Pulvertaft, R. J. V. 1965. A study of malignant tumours in Nigeria by short-term tissue culture. J. Clin. Path. 18:261.
5. Klein, E., G. Klein, J. S. Nadkarni, H. Wigzell, and P. Clifford. 1968. Surface IgM-Kappa specificity on a Burkitt lymphoma cell in vivo and in derived culture lines. Cancer Res. 28:1300.
6. Finegold, I., J. L. Fahey, and H. Granger. 1967. Synthesis of immunoglobulins by human cell lines in tissue culture. J. Immunol. 99:839.
7. Levy, J. A., M. Virolainen, and V. Defendi. 1968. Human lymphoblastoid lines from lymph node and spleen. Cancer. 22:517.
8. Lerner, R. A., and L. D. Hodge. 1971. Gene expression in synchronized lymphocytes: studies on the control of synthesis of immunoglobulin polypeptides. J. Cell. Physiol. 77:265.
9. Nilsson, U. R., and H. J. Müller-Eberhard. 1965. Isolation of B1γ-globulin from human serum and its characterization as the fifth component of complement. J. Exp. Med. 122:277.
10. Bokisch, V. A., H. J. Müller-Eberhard, and C. G. Cochrane. 1969. Isolation of a fragment (C3a) of the third component of human complement containing anaphylatoxin and chemotactic activity and description of an anaphylatoxin inactivator of human serum. J. Exp. Med. 129:1109.
11. Clark, H. F., and C. C. Shepard. 1963. A dialysis technique for preparing fluorescent antibody. Virology. 20:642.
12. McConahey, P., and F. J. Dixon. 1966. A method of trace iodination of proteins for immunologic studies. Int. Arch. Allergy and App. Immunol. 29:185.
13. Mardiney, Jr., M. R., and H. J. Müller-Eberhard. 1965. Mouse B1γ-globulin: production of antiserum and characterization in the complement reaction. J. Immunol. 94:677.
14. Theofilopoulos, A. N., V. A. Bokisch, and F. J. Dixon. 1974. Receptor for soluble C3 and C3b on human lymphoblastoid (Raji) cells: properties and biological significance. J. Exp. Med. 139:696.
15. Nilsson, U. R., and H. J. Müller-Eberhard. 1967. Deficiency of the fifth component of complement in mice with an inherited complement defect. J. Exp. Med. 125:1.
16. Rother, K., U. Rother, H. J. Müller-Eberhard, and U. R. Nilsson. 1966. Deficiency of the sixth component of complement in rabbits with an inherited complement defect. J. Exp. Med. 124:773.
17. Ruddy, S., and K. F. Austin. 1971. C3b inactivator of man. II. Fragments produced by C3b inactivator cleavage of cell-bound or fluid phase C3b. J. Immunol. 107:742.
18. Jondal, M., G. Holm, and H. Wigzell. 1972. Surface markers on human T- and B-lymphocytes. I. A large population of lymphocytes forming nonimmune rosettes with sheep red blood cells. J. Exp. Med. 136:207.
19. Bentwich, Z., S. D. Douglas, F. P. Siegal, and H. G. Kunkel. 1973. Human lymphocyte-sheep erythrocyte rosette formation: some characteristics of the interaction. Clin. Immunol. Immunopath. 1:511.
20. Eden, A., G. W. Miller, and V. Nussenzweig. 1973. Human lymphocytes bear membrane receptors for C3b and C3d. J. Clin. Invest. 52:3239.
21. Christian, C. L. 1960. Studies of aggregated γ-globulin. I. Sedimentation, electrophoretic and anticomplementary properties. J. Immunol. 84:112.
22. Ishizaka, K. 1963. Gamma globulin and molecular mechanisms of hypersensitivity reactions. Prog. Allergy. 7:32.
23. Miller, G. W., P. H. Saluk, and V. Nussenzweig. 1973. Complement-dependent release of immune complexes from the lymphocyte membrane. *J. Exp. Med.* 138:495.

24. Ross, G. D., E. M. Rabellino, M. J. Polley, and H. M. Grey. 1973. Combined studies of complement receptor and surface immunoglobulin-bearing cells and sheep erythrocyte rosette-forming cells in normal and leukemic human lymphocytes. *J. Clin. Invest.* 52:377.

25. Schiff, R. I., R. H. Buckley, R. B. Gilbertsen, and R. S. Metzgar. 1974. Membrane receptors and in vitro responsiveness of lymphocytes in human immunodeficiency. *J. Immunol.* 112:376.

26. Glade, P., and K. Hirschorn. 1970. Products of lymphoid cells in continuous culture. *Am. J. Pathol.* 60:483.

27. Dickler, H. B., F. P. Siegal, Z. H. Bentwich, and H. G. Kunkel. 1973. Lymphocyte binding of aggregated IgG and surface Ig staining in chronic leukemia. *Clin. Exp. Immunol.* 14:97.

28. Dierich, M. P., S. Ferrone, M. Pellegrino, and R. A. Reisfeld. 1974. Characterization of C3 receptors on lymphoid cells. *Fed. Proc.* 33:759.

29. Nelson, D. S., and G. Uhlenbruck. 1967. Studies on the nature of the immune adherence receptor. I. The inhibition of immune adherence by soluble mucoids and mucopolysaccharides and by human erythrocyte ghosts. *Vox Sanguinis.* 12:43.

30. Shevach, E. M., R. Herberman, M. M. Frank, and I. Green. 1972. Receptors for complement and immunoglobulin on human leukemia cells and human lymphoblastoid cell lines. *J. Clin. Invest.* 51:1933.

31. Pincus, S., C. Bianco, and V. Nussenzweig. 1972. Increased proportion of complement-receptor lymphocytes in the peripheral blood of patients with chronic lymphocytic leukemia. *Blood J. Hematol.* 40:303.

32. Eden, A., C. Bianco, and V. Nussenzweig. 1973. Mechanism of binding of soluble immune complexes to lymphocytes. *Cell. Immunol.* 7:459.

33. Huber, H., M. J. Polley, W. D. Linscott, H. H. Fudenberg, and H. J. Müller-Eberhard. 1968. Human monocytes: distinct receptor sites for the third component of complement and for immunoglobulin G. *Science (Wash. D. C.).* 162:1281.

34. Ross, G. D., and M. J. Polley. 1974. Human lymphocyte and granulocyte receptors for the fourth component (C4) and the role of granulocyte receptors in phagocytosis. *Fed. Proc.* 33:759.