IDIOTYPIC DETERMINANTS OF IMMUNOGLOBULIN M DETECTED ON THE SURFACE OF HUMAN LYMPHOCYTES BY CYTOTOXICITY ASSAYS*

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Immunoglobulin (Ig) determinants have been detected on lymphocyte membranes of mouse, rabbit, and man with the use of anti-Ig sera by immunofluorescence, radioimmunoassay, and radioautography. In the mouse the complement-mediated cytotoxicity assay has proved to be effective for detection of such Ig determinants (1). However, despite some limited observations in man (2, 3), most workers have failed to make this valuable procedure work with human lymphocytes (4). Since the dominant Ig on lymphocytes appears to be IgM (5), it was thought to be of special interest to study the lymphocytes of individuals with unusual IgM proteins in their serum. The ready elicitation of idiotypic antisera to these proteins made it possible to employ these antisera for identification of similar proteins on the lymphocyte membranes.

This report describes the successful use of the microdroplet cytotoxicity assay (6) in detecting surface immunoglobulins on peripheral blood lymphocytes of healthy persons, one patient with macroglobulinemia, and one patient with a monoclonal IgM cold agglutinin with anti-I specificity (7). Idiotypic IgM determinants similar to those on the serum proteins were demonstrated on a proportion of the lymphocytes of these two patients.

Materials and Methods

Lymphocytes were isolated from heparinized venous blood of four healthy persons and of two untreated patients with monoclonal IgM serum proteins. The protein of one of the patients, Phi, was a γM kappa cold agglutinin with anti-I specificity (7); that of the second patient, Sto, was also a γM kappa protein with no known specificity. The entire purification of the lymphocytes was carried out at 37°C by incubation on nylon columns for 30 min followed by Ficoll gradient centrifugation (8). The cells (>98% small lymphocytes) were washed four times with phosphate-buffered saline, pH 7.2, and were immediately tested.

For the cytotoxicity tests the isolated lymphocytes were suspended in McCoy's medium and adjusted to 10^6 cells/ml. A modification of the microdroplet method (6) was used.

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1 μl of antiserum was reacted with 1 μl of cell suspension for up to 15 min at room temperature and for 15 min at 37°C. Then 5 μl of rabbit serum (Pel-Freez Biologicals, Inc., Rogers, Ark.) as source of complement was added and incubation for a further 3 hr at room temperature was performed. Cytotoxicity was assessed by eosin dye exclusion with the use of phase-contrast microscopy. For every quantitation a minimum of 12, but usually 36, tests was carried out and the results were expressed as their mean. Each experiment included control tests with heat inactivated normal rabbit serum (NRS) or rabbit fraction II for determination of the background killing and an anti-lymphocyte serum as a positive control (>95% killing) for the complement source.

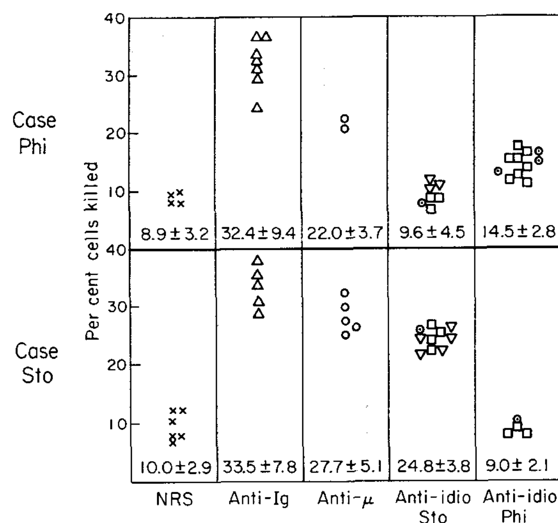

FIG. 1. Cytotoxicity tests with anti-Ig and anti-idiotypic antisera on peripheral blood lymphocytes of case Sto and of case Phi. Antisera absorbed with: soluble Ig, □; solid Ig, ⊙. Isolated anti-Sto antibody absorbed with soluble Ig, ▽. Anti-human Ig unabsorbed, △; anti-human μ absorbed with solid Ig, ○. Each point in the figure represents the mean of at least 12 single tests. The numbers given are the mean values and standard deviation.

The antisera against Sto and Phi proteins (anti-idio Sto and anti-idio Phi) were prepared as described previously (9). These were absorbed either with soluble Ig or with solid adsorbents of Sepharose beads and Ig. In addition an isolated idiotypic antibody preparation against IgM Sto was prepared by absorption of Sto antiserum on an adsorbent of Sepharose and γM Sto and elution with 0.1 M HCl.

RESULTS

Cytotoxicity of Lymphocytes When Tested with Anti-Human Ig and Anti-Human μ Sera.—In tests with an anti-human Ig serum, the mean percentage of killed cells was 30.1 ± 8.5% in healthy controls and 32.4 ± 9.4 and 33.5 ± 7.8% in cases Phi and Sto, respectively (Fig. 1). The mean background cytotoxicities in the presence of NRS and rabbit fraction II were of the order of 10%. The mean effective cytotoxicity after subtraction of background killing

with NRS was 21.3% in healthy controls and 23.4 and 23.5%, respectively, in the patients (Table I).

With an anti-human μ antiserum, 9.7% mean effective cytotoxicity was observed in healthy controls and 13.0 and 17.7% with cases Phi and Sto, respectively (Table I). A rise to 44% occurred in case Sto in later bleedings.

Cytotoxicity of Lymphocytes Tested with Idiotypic Antisera. —Each idiotypic antiserum produced effective cytotoxicity with the lymphocytes of the homologous patient only (Table I). Approximately 6% of the homologous lymphocytes were killed specifically with anti-idio Phi. With anti-idio Sto about 15% and later as high as 42% cytotoxicity was obtained with homologous lymphocytes. With case Sto in whom higher anti-μ cytotoxicity values were obtained

TABLE I

Mean Cytotoxicity Values as Per Cent Killed after Subtraction of Background Killing with Normal Rabbit Serum

Cells	Anti-human Ig	Anti-human μ	Anti-idio Sto	Anti-idio Phi
Healthy controls	21.3	9.7	0.4	0.7
Patients				
Phi	23.4	13.0	0.6	5.5
Sto I*	23.5	17.7	14.8	0
Sto II‡	49.0	44.0	42.0	0

* Mean values of bleedings Jan.–Apr. 1972.
‡ Mean values of bleedings in May 1972.

than in case Phi, the idiotypic cytotoxicity values approached the anti-μ values. This is particularly evident in the later tests.

Three antisera, anti-human Ig, anti-human μ, and anti-idio Sto, were tested with normal lymphocytes and Sto lymphocytes for their cytotoxic ability in the undiluted state, at 1:4 and 1:16 dilution (Fig. 2). The specificity of the anti-idio Sto for Sto lymphocytes is again shown. With increasing antibody dilution a relatively fast decrease of cytotoxicity was observed. Cytotoxicity results were not obviously affected by absorption of the antisera with soluble or solid adsorbents (Fig. 1). Preincubation (for 5 hr at 37°C) of the lymphocytes of a group O healthy person with serum of each patient and the subsequent testing of washed lymphocytes gave negative results with both of the anti-idiotypic antisera.

DISCUSSION

These results show that the complement-mediated lymphocytotoxicity test can detect surface Ig determinants on a proportion of peripheral blood lymphocytes in man in the same way as in other species. By this technique 21.3% of the cells of healthy persons were killed by an anti-Ig serum with μ, γ, kappa,

and lambda specificity. This value is in accord with the range of 9–32% (mean 22.9%) Ig-bearing cells detected among normal human peripheral blood lymphocytes by immunofluorescence (10). The mean cytotoxicity obtained in healthy persons with an anti-human μ was 9.7%, and this compares well with mean values of 9.3 and 13% IgM positive cells by immunofluorescence (10, 11). The high background killing of approximately 10% of cells represents a limitation of the technique. In case Phi with cold agglutinin disease the cytotoxicity values of 23.4 and 13.0%, respectively, with anti-Ig and anti-μ sera were not appreciably above the mean values in healthy persons (Table I). In case Sto a dramatic rise in cytotoxicity with anti-Ig and anti-μ occurred during a

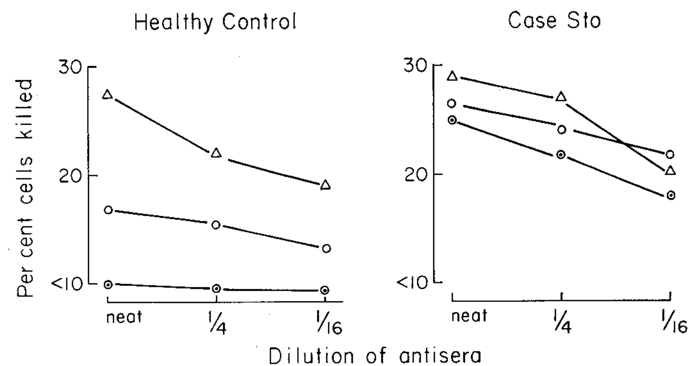

FIG. 2. Effect of dilution of antisera in cytotoxicity tests with anti-human Ig, △; anti-human μ absorbed with solid Ig, ○; and with an idiotypic antiserum against macroglobulin Sto (absorbed with solid γM), ⊙. Each point represents a mean of 12 tests.

period of observation of 5 months. In this patient the proportion of cells reacting with anti-μ closely approximated the proportion reacting with the polyvalent anti-Ig serum especially in the later tests (Table I).

The cytotoxicity achieved with Phi lymphocytes and the idiotypic antiserum against Phi protein was less than half of the total value with anti-μ. A much larger proportion, up to 96%, of cytotoxicity relative to anti-μ values was obtained with Sto cells and anti-idio Sto. It seems likely that the majority of immunoglobulin-bearing peripheral blood lymphocytes of case Sto have surface γM of the same idiotype as that of his serum macroglobulin peak. Control experiments indicated that the positive cytotoxicity especially with the idiotypic antisera was not due to the presence of passively adsorbed serum macroglobulin onto lymphocytes. In recent lymphocytotoxicity experiments evidence was obtained suggesting that "cross idiotypic specificity" determinants can be detected on lymphocytes of patients with anti-I and anti-i cold agglutinin disease with the use of idiotypic antisera against anti-I and anti-i cold agglutinins.

For the successful performance of the complement-mediated cytoxicity test

the incubation times, the source of complement, and the antibody concentration are important factors. Rabbit serum as a source of complement was found to be more effective than guinea pig serum in cytolysis of mouse and human lymphocytes by alloantibody. This may possibly be due to its content of a natural antibody which may enhance the binding of complement by the alloantibody (12). Recent experiments in this laboratory with human peripheral blood lymphocytes and anti-Ig sera with and without sublytic dilutions of an anti-human lymphocyte serum (ALS) have shown that more reproducible results are obtained in the presence of ALS. The concentration of specific antiserum also was important in obtaining cytotoxicity. Too high concentrations of antibody can show a prozone effect (13) and too low concentrations will fail to give cytotoxicity. The optimal amount of undiluted antisera to be used in the present studies were first determined (Fig. 2).

Parallel studies were carried out on the lymphocytes of these patients by cytotoxicity as well as immunofluorescence. Similar results were obtained although the fluorescence work with the anti-idiotype antisera gave less consistent results. Further evidence for the similarity of the surface and serum IgM proteins was obtained in patients with cold agglutinin disease through identification of cold agglutinins on the lymphocytes by the formation of I or i red cell rosettes in the cold; the rosettes had the same specificity as the serum proteins. These studies will be reported separately. Recently Preud'homme and Seligmann have obtained evidence from fluorescent antibody studies for increased IgM-bearing lymphocytes in cases of macroglobulinemia; kappa and lambda restriction was also observed (11). All of these findings lend support to the hypothesis that precursors of cells that actively secrete antibody have on their surface antigen receptors that are similar, if not identical to, the antibody molecules eventually secreted by them. However, the possibility that some of these cells are also secreting the IgM protein is not ruled out.

SUMMARY

The complement-mediated cytotoxicity assay has been used to demonstrate surface immunoglobulin determinants on human peripheral blood lymphocytes. In two individuals with monoclonal serum IgM components, idiotypic antisera demonstrated similar components on a significant proportion of the peripheral blood lymphocytes.

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