Bone marrow-derived (B) lymphocytes in man and mouse have characteristic cell surface markers, such as immunoglobulin, that distinguish this subpopulation from other types of lymphocytes (1–4). In the mouse, thymus-derived (T) lymphocytes also have cell markers, such as theta antigen (Thy 1.2) and Ly antigens, that typify this cell type (5–7). In contrast, human T-cell surface markers are not as well delineated. T lymphocytes have receptors for and form rosettes with unsensitized sheep erythrocytes (SRBC) and display a differentiation marker, human T lymphocyte antigen (HTLA) (8–10). The chemical nature of both the SRBC receptor and HTLA are not well defined.

We have previously shown that a protein, termed macromolecular insoluble cold globulin (MICG), is an exclusive product of T cells in the mouse (11-13). The name MICG has been used to describe the major physicochemical properties of this protein. It has a mol wt of 225,000 daltons, is insoluble in the cold in nonionic detergents, and migrates as a β-globulin on electrophoresis. The present communication describes the characterization of an analogous protein in human T cells, termed T-MICG. We further demonstrate a protein in null (N) cells, termed N-MICG, which has similar physical properties to, but differs antigenically from, T-MICG.

Materials and Methods

Isolation of Human Lymphocytes and Subpopulations. Peripheral blood mononuclear cells (PBL) were separated from 100 ml of heparinized whole blood by Ficoll-Hypaque centrifugation (FHC) (14). The PBL were depleted of monocytes by adherence to plastic Petri dishes for 1 h at 37°C (15). Morphological examination of these cells (Wright-Giemsa staining of smears) demonstrated a mean of 98% lymphocytes and 2% monocytes. They are subsequently referred to as lymphocytes. T cells in these preparations were enumerated by rosette formation with SRBC and separated from PBL by rosette sedimentation (9, 16). Briefly, fresh SRBC (Grand Island Biological Co., Grand Island, N. Y.) are washed in phosphate-buffered saline (PBS) and resuspended to a concentration of 5% in Hanks’ Balanced Salt Solution (HBSS) (Grand Island Biological Co.). 1 ml of neuraminidase (Behring Diagnostics, Somerville, N. J.), diluted to 0.5 U/ml in HBSS, is added to 1 ml of 5% SRBC. The mixture is incubated for 30 min at 37°C,
washed with RPMI-1640 (Grand Island Biological Co.), and diluted to 0.5% SRBC. To a 0.5-
ml suspension of 0.5% SRBC is added 0.5 ml of a lymphocyte suspension containing 2 × 10^6
cells/ml. The mixture is incubated at 37°C for 15 min, centrifuged at 200 g for 5 min at room
temperature, and placed at 4°C for 60 min. The tubes are then inverted and the cells stained
with 0.5% toluidine blue and placed in a hemocytometer and 200 lymphocytes counted and
expressed as the percent of E rosettes, i.e., percent of lymphocytes forming rosettes with SRBC
(three or more). After PBL were rosetted with SRBC, the cell mixture was subjected to a second
FHC. The pellet, containing free SRBC and E rosettes, was incubated at 37°C for 15 min to
dissociate the rosettes and then either hypotonically lysed in deionized water or subjected to
another FHC. These cells are referred to as T-lymphocytes (E^+). This subpopulation contained
less than 2% surface immunoglobulin-positive cells (SIg^+), and 97% of these cells formed rosettes
with SRBC, i.e., E^+. The lymphocyte fraction at the Ficoll-medium interphase of the second
FHC was rerosetted with SRBC and subjected to a third FHC. Unrosetted cells at the Ficoll-
medium interphase of this last separation were referred to as B-enriched lymphocytes and
contained 70–80% SIg^+ cells and less than 2% E^+ cells. Fluorescent microscopy, using fluorescein
isothiocyanate (FITC) conjugated rabbit anti-human-Fab (Behring Diagnostics) was performed
to identify SIg^+ cells, i.e., B cells (17). B cells were also enumerated by rosetting with SRBC
coated with antibody (IgM) and complement (EAC) (18). To enumerate lymphocytes forming
rosettes with EAC, 0.5 ml of PBL (2 × 10^6/ml) was added to 0.5 ml of 0.5% EAC. This mixture
was incubated for 30 min at 37°C with agitation. The rosettes formed were quantitated as
above. B-enriched lymphocytes quantitated by this technique were 70–75% EAC positive.

Purified B lymphocytes were isolated utilizing rabbit anti-human-Fab columns as described
by Chess et al. (19, 20). In this procedure, isolated antibody to human Fab is linked to cyano
g bromide activated Sephadex G-200. The cells (200 × 10^6) applied to this column were either
PBL or B-enriched cells from the Ficoll-medium interphase after E-rosette sedimentation. In
the case of PBL, unbound cells, consisting of T (E^+) and N (SIg^- and E^-) cells are collected and
bound cells eluted by competitive inhibition with human IgG (21). The bound-cell fraction
contains 98% SIg^+ cells and is referred to as B cells, while the unbound cells contain 82% E^+
cells and < 2% SIg^+ cells.

Isolation of N cells from PBL and B-enriched lymphocytes is described in the text.

**Cell Culture Conditions for Protein Synthesis in Human Lymphocytes.** Single cell suspensions of
PBL, T cells, B cells, or N cells (10–50 × 10^6) were incubated in 3 ml of Eagle’s minimal
essential medium minus leucine (Grand Island Biological Co.), without fetal calf serum, with
10 μCi of [14C]leucine, sp act 298 mCi/mmol, (New England Nuclear, Boston, Mass.) as
previously described (12). The cells were incubated for 4 h at 37°C in a mixture of 95% air-5%
CO_2 in a humidified atmosphere (22, 23). After incubation, cell viability was determined with
0.05% (vol/vol) trypan blue in PBS and only cultures with greater than 95% viability further
utilized. The radiolabeled cells were then immersed in ice water, made 0.06 M in iodoacetamide,
and centrifuged at 400 g for 10 min. The cells were washed in Eagle’s complete medium four
times at 4°C, resuspended in isotonic buffer (22), containing 0.5% Nonider (NP-40) (Particle
Data Lab. Ltd., Elmhurst, Ill.), 0.06 M iodoacetamide and 1,000 U/ml of Trasylol (Calbiochem,
San Diego, Calif.). Detergent lysis was allowed to occur at 4°C for 20 min, and the resultant
cell lysate centrifuged at 105,000 g for 30 min at 4°C, and the pellet discarded.

**Isolation of MICG by Cold Precipitation.** Cell lysates were incubated for 2 days at 4°C,
centrifuged at 25,000 g for 20 min, and the precipitate vigorously washed three times in cold
PBS. These conditions were found to be optimal for cold precipitation (12). The precipitate
was solubilized for 30 min at 37°C either in 2% (wt/vol) sodium dodecyl sulfate (SDS)-8 M
urea-0.06 M iodoacetamide-Trasylol (1,000 U/ml)-0.1 M Tris buffer pH 9.2 (SDS-urea buffer)
or in 0.5% Triton-X-100 (Amersham-Searle, Arlington Heights, Ill.)-2 M urea-0.1 M Tris buffer
pH 8.2 (Triton-urea buffer).

**Preparation of Antisera.** New Zealand white rabbits were used to raise all antisera (12).
Homogenous T-MICG, N-MICG, or a combination of T and N-MICG was prepared by cold
precipitation of isolated T and/or N-cell lysates. Homogeneity was assessed by SDS-polyacryl-
amide gel electrophoresis (SDS-PAGE) and gel filtration on a Biogel A-5M column (0.9 cm
× 90 cm) equilibrated with 1% SDS-buffer (12). The isolated proteins used for immunization
were solubilized in Triton-urea buffer. Anti-serum to human IgM, IgA, IgG, albumin, transferrin,
and α2 macroglobulin have been previously described (24, 25). These antisera were
monospecific by immunoelectrophoresis and Ouchterlony analysis using normal human plasma and serum as antigen, as well as concentrated fractions of isolated serum proteins. In addition, specificity of antisera was shown by radioimmunoassay (1). All antisera were heat inactivated at 56°C for 30 min before use. The antisera are referred to in the text as anti-T-MICG, i.e., antisera to T-cell derived MICG (T-MICG); anti-N-MICG, i.e., antisera to N-cell derived MICG (N-MICG); or anti-(T + N)-MICG, i.e., a single antisera to both T and N-cell derived MICG.

Radiiodination of Lymphocyte Lysates. Cold precipitates of PBL lysates were deliberately not washed, to obtain contamination of the cryoprecipitate with other lymphocyte associated proteins. The contaminated precipitate was dissolved in 0.5% Triton-2 M urea-Tris buffer pH 8.1. The dissolved proteins were then radioiodinated with 125I (New England Nuclear) by the Chloramine T method as previously described (26, 27). An aliquot of this sample was directly electrophoresed or immune precipitated before SDS-PAGE.

Immune Precipitation. Radi iodinated samples were immune precipitated with antisera to MICG (250 µl) in antibody excess and incubated at 37°C for 16 h. The precipitates were washed three times in cold PBS, dissolved in SDS-urea buffer, and incubated at 37°C for 30 min. In some experiments, radioiodinated lysates were precipitated with anti-T-MICG and the supernate of this precipitate divided into two equal aliquots. One portion was treated with anti-T-MICG and the other with anti-N-MICG. In these experiments the first antisera always depleted the protein to which it was directed.

SDS-PAGE. Solubilized cold and immune precipitates were reduced with 0.5 M 2-mercaptoethanol for 1 h at 37°C, alkylated with 0.65 M iodoacetamide, and then dialyzed against 0.01 M phosphate buffer pH 7.0, 0.1% with respect to SDS (0.1% SDS buffer). The samples were electrophoresed on 5% PAGE in 0.1% SDS for 3 h at 8 mA per gel (28). The gels were frozen at -70°C and sliced into 1-mm sections and the radioactivity eluted overnight at 37°C in 0.1% SDS-buffer. 5 ml of Aquasol (New England Nuclear) was added after the overnight incubation and the samples counted in a liquid scintillation spectrometer. Marker proteins for molecular weight determination of samples were simultaneously electrophoresed on companion gels and stained (12). Alternatively, IgM and IgG were isolated by immune precipitation of [14C]leucine labeled mouse plasmacytoma cells of the appropriate class. The radiolabeled markers were reduced and electrophoresed on companion gels, which were sliced and counted (12). Intracellular 8 S IgM (200,000 daltons) was immunoprecipitated from MOPC 104E labeled lysates and electrophoresed in the unreduced state. Standard curves for molecular weight were constructed for each electrophoretic run and the values given are means of at least three separate determinations.

Cytotoxicity. Lymphocyte cell suspensions were washed in McCoy's medium (Grand Island Biological Co.) and cytotoxicity measured in microtiter plates employing eosin dye incorporation to determine cell death (29, 30). To 2,000 cells in 1 µl of McCoy's medium containing 5% fetal calf serum was added 1 µl of rabbit antisera or normal rabbit serum. The cells were incubated at room temperature for 30 min and then 5 µl of rabbit complement (Grand Island Biological Co.) was added to each well and incubation continued for 2 h at room temperature. Eosin dye was added and each well scored for the number of cells incorporating dye (cell death) in the following manner: 1 = 0-10%; 2 = 11-20%; 3 = 21-30%; 4 = 31-50%; 5 = 51-70%; 6 = 71-80%; 7 = 81-90%; and 8 >91%. Controls in these experiments consisted of normal rabbit serum and rabbit anti-human albumin antisera, or medium. These sera showed no cytotoxicity in the presence or absence of rabbit complement. The rabbit complement did not induce cell death when incubated with lymphocytes in the absence of antiserum.

Immunofluorescence of Lymphocyte Plasma Membrane. Indirect immunofluorescence was performed on viable PBL and subpopulations obtained from anti-Fab columns (31, 32). Lymphocytes (2 × 10⁶) were incubated for 1 h at 37°C in 3 ml Eagle's minimal essential medium supplemented with 20% fetal calf serum and 0.2% sodium azide. The cells were washed, resuspended in 0.1 ml of medium, and incubated at 4°C for 30 min with 0.1 ml of anti-(T + N)-MICG antiserum (1:64). The antiserum employed was the IgG fraction, heat inactivated at 56°C and adsorbed with calf liver powder before use. In some experiments the rabbit IgG was digested with pepsin to produce an Fab2 fragment, as previously shown (33). The cells were washed three times in medium, resuspended in 0.1 ml of medium, and 0.1 ml of FITC conjugated goat anti-rabbit IgG (Behring Diagnostics) (1:32) added. Incubation was continued
for 30 min at 4°C, the cells washed three times, and finally resuspended in 0.1 ml of medium. A drop of the cell suspension was applied to a microscope slide with the bottom of a 12 x 75 mm test tube, fixed in ethanol (95%) and a cover slip applied in buffered glycerol (34).

Direct immunofluorescence was also performed on viable lymphocytes utilizing FITC conjugated rabbit anti-human-Fab antiserum (Behring Diagnostics). Lymphocytes, 2 x 10^7/0.1 ml of medium, were incubated with a 1:16 dilution of antiserum for 30 min at 4°C and processed as in the indirect technique.

The preparations were examined with a Zeiss IV FL-epifluorescent microscope equipped with an Osram 12 V, 100 W, halogen lamp and an incident-light fluorescence illuminator. The following combination of exciting and barrier filters and of beam splitters were used: for visualization of fluorescein, exciting filter 450-490 nm, barrier filter LP 520, beam splitter FT 510. The same field of cells was also examined by phase contrast microscopy. Photographs were recorded on Ektachrome ASA 200 film. Indirect specificity controls consisted of normal rabbit serum, in place of anti-(T + N)-MICG, and absorption experiments. Less than 1% PBL were immunofluorescent positive at 1:2 dilution of normal rabbit IgG (1 mg/ml). Indirect fluorescence was completely blocked by absorption of anti-(T + N)-MICG with either PBL, T + N cells (300 x 10^6/0.5 ml of antibody) or T + N-MICG antigen (45 #g). Indirect immunofluorescence was also blocked by adsorption of FITC goat anti-rabbit IgG with rabbit IgG. There was no inhibition of immunofluorescence when anti-(T + N)-MICG antiserum was adsorbed with lyophilized human plasma (20 mg/ml), normal B cells, or chronic lymphocytic leukemia cells at cell concentrations of 300 x 10^6/0.5 ml of antibody.

**Results**

**Isolation and Characterization of MICG Proteins from Human Lymphocytes.** When radiolabeled lysates from PBL were cold precipitated, SDS-PAGE demonstrated two macromolecules with mol wt of 225,000 and 185,000 daltons, accounting for 90 and 10%, respectively, of the cryoprecipitate (Fig. 1 a). Incubation of cell lysates for 48 h at 22°C did not induce the precipitation of MICG-like proteins. Similar to mouse MICG, both macromolecules had the electrophoretic mobility of β-globulins (12). Cryoprecipitate analyzed by SDS-PAGE, and stained for protein with Coomasie Blue is shown in Fig. 2 a. Using suitable standards, the mol wt of the two major proteins were calculated to be 225,000 and 185,000 daltons, similar to the radiolabeled preparations. Although a small amount of contamination is present in this gel, a homogenous protein can be obtained by thorough washing of the cold precipitate.

Analysis of cold precipitates from T and B-enriched cells, isolated by rosette sedimentation, showed that the T lymphocytes synthesized the 225,000 dalton protein, while the B-enriched cells synthesized the 185,000 dalton macromolecule (Figs. 1 b, 1 c and 2 b, 2 c). In four separate experiments with different histocompatibility typed lymphocytes, neither cell population synthesized the two proteins simultaneously. To investigate whether some form of interaction between T and B cells might influence the relative quantity of the two proteins synthesized, varying numbers of T cells were added to a constant number of B cells before incubation with [14C]leucine. SDS gels of the cryoprecipitates from this experiment demonstrated that the amount of either the 225,000 or 185,000 dalton protein was similar to that obtained from equal numbers of isolated T or B cells.

**Further Purification of B Lymphocytes.** The B-cell enriched fraction, obtained by double rosette sedimentation depletion of T cells, contained 70–80% SIg⁺ cells, 3–7% monocytes, and 10–15% lymphocytes which were E⁻ and SIg⁺. To obtain a more homogeneous population of B-lymphocytes, PBL were separated into subpopulations by fractionation on anti-human-Fab Sephadex G-200 columns. Utilizing this technique along with monocyte depletion by adherence to plastic Petri dishes, 98% SIg⁺
cells were obtained. Cold precipitated lysates from the unbound cell fraction (T and N) synthesized both the 225,000 and the 185,000 dalton macromolecules (Fig. 3a), while equal numbers of viable B lymphocytes from the anti-Fab column did not synthesize either protein (Fig. 3b). To be certain that these B lymphocytes were capable of protein synthesis, radiolabeled lysates were shown to synthesize IgM when analyzed on SDS-PAGE (not shown).

Although the T cells obtained by the anti-Fab columns contain 80–85% E+ cells and less than 2% SIg+ cells, there is a substantial number of cells that are E− and SIg−, referred to as N cells (21). To establish whether the 185,000 dalton macromolecule was the product of this N-cell population, unbound cells from the anti-Fab column were rosetted with SRBC and subjected to FHC. The radiolabeled lymphocytes from the SRBC pellet (98% E+) were lysed, cold precipitated, and subjected to SDS-PAGE. These T cells again demonstrated the 225,000 dalton component, while the N cells, at the Ficoll-medium interphase, synthesized the 185,000 dalton protein (Fig. 4a and 4b). The N cells consisted of less than 2% E+ cells and less than 1% SIg+ lymphocytes.

Further proof of the N-cell origin of the smaller protein was obtained in the
FIG. 2. Cold precipitation of human MICG protein from lysates of: a, PBL (300 x 10⁶); b, T cells (75 x 10⁶); c, B cells (75 x 10⁶). Precipitates were reduced and subjected to 5% SDS-PAGE and stained with 0.5% Coomassie Blue.

following experiment. PBL were subjected to double rosette sedimentation with SRBC to deplete the T-cells. An aliquot of the Ficoll-medium interphase cells was radiolabeled and the remainder applied to anti-human-Fab columns. Bound and unbound cells were also radiolabeled and all lysates cold precipitated and analyzed on SDS-PAGE. The interphase cells, i.e., B enriched cells, demonstrated a single peak of radioactivity of 185,000 daltons. The unbound, N cells, synthesized a radioactive peak of the same mobility, while no cold insoluble radioactive peak was found in the eluted B-cell fraction. The N cells were less than 2% Slg⁺ and less than 1% E⁺. These two proteins are referred to as T-MICG (225,000 daltons) and N-MICG (185,000 daltons). The same sequence of experiments, performed on lymphocytes obtained from three other individuals, yielded identical results to those above.

Immunological Analysis of Human Cryoproteins. On immunoelectrophoresis anti-(T + N)-MICG antiserum showed two precipitin arcs of β-mobility against PBL lysates, but did not react with whole human plasma or serum. This antiserum showed single precipitin arcs against purified T-MICG and purified N-MICG. Similar results were obtained in Ouchterlony analysis. Antiserum to purified T-MICG gave a single
Fig. 3. Cold precipitation of radiolabeled lysates derived from T and B cells, isolated by anti-human-Fab columns. PBL were subjected to an anti-human-Fab column and unbound (T and N) cells; a, and eluted (B) cells; b, (22 x 10^6 cells) labeled with [3H]leucine. Cold precipitates were reduced and subjected to SDS-PAGE (5%).

Fig. 4. Rosette sedimentation on FHC of the unbound cell fraction from anti-human-Fab column. PBL were subjected to anti-human Fab columns and the unbound fraction incubated with SRBC and rosette sedimented by FHC. a, SRBC pellet (T cells) was hypotonically lysed and the lymphocytes (30 x 10^6) radiolabeled with [3H]leucine. b, Ficoll-medium interphase cells (null cells) (30 x 10^6) were also radiolabeled with [3H]leucine. Radiolabeled lysates were cold precipitated, reduced, and subjected to 5% SDS-PAGE.
precipitin line against purified T-MICG, but did not react with purified N-MICG. With anti-N-MICG antiserum, there was a single precipitin line against purified N-MICG, but no reaction with purified T-MICG. When each of these antisera was reacted with (T + N)-MICG, only one or the other of the two precipitin lines seen with anti-(T + N)-MICG developed. Rabbit anti-whole human serum did not react with isolated T-MICG or N-MICG, nor did antiserum to human IgG, IgA, IgM, IgD, IgE, α2 macroglobulin, transferrin, albumin, or cold insoluble globulin.

When an unwashed cold precipitate from PBL is subjected to SDS-PAGE and stained for protein, a number of bands are seen, in addition to T-MICG and N-MICG (not shown). In contrast, thorough washing of the cold precipitate, before dissolving for SDS-PAGE, reveals only two bands (Fig. 2a). The other protein bands, deliberately produced by incomplete washing of the cryoprecipitate, would serve as a control for determining the specificity of the antisera. Accordingly, unwashed precipitates from PBL were dissolved in Triton-urea and radioiodinated. The radiolabeled proteins were immune precipitated with one antiserum and the supernate tested for reactivity with a second antiserum. The precipitates were analyzed on SDS-PAGE. The results in Fig. 5 demonstrate that antiserum to T-MICG only precipitated the larger (225,000 daltons) of these two proteins (Fig. 5a), while antiserum to N-MICG only reacted with the smaller (185,000 daltons) protein (Fig. 5b). Furthermore, anti-(T + N)-MICG antiserum precipitated both of these components from the radiolabeled lysates (Fig. 5c). In these studies, the first antiserum totally depleted the supernate of the component to which it was reactive. Normal rabbit serum had no effect on these radioiodinated proteins (Fig. 5c).

**Cytotoxicity Induced by Antiserum to MICG.** The expression of these antigens on the cell surface was examined by means of antibody induced cytotoxicity. In the experiments shown in Fig. 6, PBL, or cells fractionated on anti-Fab columns were reacted with dilutions of antiserum in the presence of complement. Anti-T-MICG antiserum, to a titer of 1:256, induced 80% cytotoxicity of PBL, while anti-N-MICG and normal rabbit serum were not cytotoxic to PBL, beyond a titer of 1:4. When unbound cells (T + N), from anti-Fab column fractionated PBL, were treated with anti-(T + N)-MICG antiserum, to a titer of 1:128, 98% of the cells were killed (Fig. 6). In contrast, anti-(T + N)-MICG was not cytotoxic, beyond 1:2, to B cells eluted from the anti-Fab column.

**Cell Surface Distribution of MICG.** Immunofluorescent studies employing anti-(T + N)-MICG antibody revealed that 85–88% of PBL displayed a ringed fluorescent pattern (Fig. 7). If the first antibody in indirect immunofluorescence was rabbit anti-human IgM, 12% of PBL were positively stained. Similar numbers of Slg* cells were found with the direct technique. It was also shown that the Fab2 γ-fragment of anti-(T + N)-MICG antibody stained 85% of PBL in a ringed fluorescent pattern. Furthermore, anti-T-MICG antiserum stained 78–80% of PBL. The anti-(T + N)-MICG pattern was completely inhibited by absorption with the unbound cell fraction (T + N) from an anti-Fab column, as well as by isolated T and N-MICG antigen. There was no loss of immunofluorescence when the antibody was adsorbed with purified B cells, or lyophilized human plasma. Further evidence for the absence of either T or N-MICG antigen from the surface of B cells was provided using anti-Fab column purified lymphocytes. The unbound fraction demonstrated uniform ringed fluorescence (98–100%) with anti-(T + N)-MICG antibody, while only 1–2% of these
Fig. 5. Antigenic properties of human T-MICG and N-MICG. Cryoprotein (unwashed) from PBL lysates was dissolved in 0.5% Triton-X-100-2 M urea and radioiodinated. The proteins were immune precipitated at 37°C with anti-T-MICG or anti-N-MICG antiserum and the supernate treated with the second antisera. All samples were reduced and applied to SDS-PAGE (5%). a, Precipitated with anti-T-MICG (——) and the supernate treated with anti-N-MICG (-----); b, Precipitated with anti-N-MICG (——) and the supernate reacted with anti-T-MICG (-----); c, Reacted with normal rabbit serum (-----) and the supernate reacted with anti-(T+N)-MICG antiserum (——).
Fig. 6. Cytotoxicity of human lymphocytes treated with antisera to MICG proteins. PBL, T + N cells, and B cells were treated with dilutions of antiserum and complement and cell death determined by eosin dye incorporation. NRS, normal rabbit serum.

Fig. 7. Plasma membrane distribution of MICG determined by indirect immunofluorescence. PBL were reacted with rabbit anti-human-(T + N)-MICG antibody for 30 min at 4°C. The cells were washed and treated with FITC-conjugated goat anti-rabbit Ig antibody for 30 min at 4°C. After washing, a drop of cell suspension was fixed on slides and examined under a Zeiss epifluorescent microscope with selective filters.
cells were stained with FITC conjugated anti-IgM antibody. The bound fraction had 4% fluorescent-positive cells with anti-(T + N)-MICG antibody, while 97% of these cells were SIg⁺.

Discussion

This paper details observations of a human protein, MICG, which is synthesized in human T cells. The name T-MICG was derived from its major biochemical characteristics: the protein is a macromolecule with a mol wt of 225,000 daltons, is insoluble in the cold in nonionic detergents, has the mobility of a β-globulin on electrophoresis and is synthesized by T cells. We have previously applied the term MICG to a similar protein found in mouse T cells (12, 13). Preliminary evidence suggests cross-reactivity between T-MICG from mouse and human lymphocytes. We have further described another macromolecule, N-MICG, which has a mol wt of 185,000 daltons, appears to be antigenically distinct from T-MICG and is synthesized in N Cells. The term N-MICG has been employed, because the physicochemical properties of this protein are similar to T-MICG.

Evidence for the divergent cell origin of these two proteins was provided by cell separation techniques utilizing E rosette sedimentation. This revealed that the T cells synthesized the 225,000 dalton protein, while the B-enriched fraction produced the 185,000 dalton macromolecule. Analysis of cell homogeneity revealed that the T cells were 97% E⁺, while the B-enriched cells contained 75% SIg⁺ cells. When the B cells obtained by rosette sedimentation were applied to anti-Fab columns, the 185,000 dalton protein was found to be a product of the N cells and not the B lymphocytes. In these latter cell preparations, the B cells were 98% SIg⁺, while the N cells contained less than 2% SIg⁺ cells and less than 1% E⁺ cells (SIg⁻, E⁻). Furthermore, when PBL were applied directly to anti-Fab columns, neither form of MICG was synthesized in the eluted B cells. In contrast, the unbound cells, containing T and N cells, synthesized the two components. When this latter cell fraction was rosette sedimented the 225,000 dalton component was synthesized in the T cells and the 185,000 dalton component synthesized in the N-cell fraction.

Although the cell of origin of T and N-MICG differed, it remained to be determined whether these proteins were antigenically dissimilar. The evidence obtained by Ouchterlony analysis suggested, but did not prove, antigenic diversity. Further support of their antigenic uniqueness was established by utilizing radioiodinated, partially purified T and N-MICG. These experiments showed that antiserum to T-MICG precipitated the larger macromolecule (225,000 dalton), while the smaller (185,000 dalton) component remained in the supernatant. On the other hand, antiserum to N-MICG precipitated the smaller component, while the larger protein remained in the supernatant. During early studies with human MICG, we were unable to resolubilize the cryoproteins in aqueous buffers, including those containing 6 M quanidine or 8 M urea, regardless of reduction and alkylation. MICG was also not completely soluble in either NP-40 or Triton X-100 at concentrations from 0.1% to 5% (vol/vol), i.e., only 30% of the cryoprotein was soluble in 0.5% Triton X-100. The marked insolubility of these proteins in cold solutions necessitated resolubilization in 0.01% SDS or 0.5% Triton-2 M urea in which 100% of the cryoprotein was soluble. Furthermore, partially purified preparations were used and therefore monospecificity of antiserum could not be completely established. However, recent experiments,
addressing this question, have clearly demonstrated the antigenic dissimilarity of T and N-MICG.

Studies employing antibody induced cytotoxicity suggested the presence of T and N-MICG on the lymphocyte plasma membrane. Cytotoxicity experiments utilizing anti-T-MICG antiserum demonstrated that T-MICG was present on the plasma membrane of 80% of PBL. The lack of effect on antiserum to N-MICG on PBL may have been due to the small number of N cells, i.e., 4–10% in PBL. In immunofluorescent experiments, to be separately reported, we have shown that N-MICG is present on the plasma membrane of 4–7% of PBL, which are E− and Slg−. When cytotoxicity of T and N cells was induced by anti-(T + N)-MICG the percent killed cells was 98%, compared to 80% for PBL. This difference can be accounted for by the absence of B cells in the T and N-cell fraction. Furthermore, this antiserum was not cytotoxic to B cells.

Direct evidence of the plasma membrane nature of these antigens was provided by immunofluorescence examination. These studies clearly showed that T, and probably N-MICG, were located on the plasma membrane of T and N cells, respectively, but not on B-cells. In addition, we have shown that T-MICG can be capped by incubation of PBL with anti-T-MICG antiserum for 30 min at 37°C. Although these experiments suggest that neither T-MICG nor N-MICG are found on most B lymphocytes, it is possible that one of these proteins is present on a small subpopulation of B cells. The B-cell fraction from the anti-Fab column did contain 4% MICG-positive cells. We are currently examining PBL by combined immunofluorescence with FITC conjugated anti-N-MICG antiserum and rhodamine conjugated anti-Ig antiserum to determine whether a subpopulation of B-cells has both N-MICG and Ig on their surface.

In the mouse, T lymphocytes can be clearly distinguished from other lymphocyte subpopulations by virtue of a number of well defined surface markers (5–7). Mouse T cells have Thy 1.2 antigen and Ly antigens. B cells in both man and mouse are typified by cell surface immunoglobulin (1–4). In contrast, human T cells have few well characterized surface antigens. The SRBC rosette receptor has partially been characterized, while the HTLA antigen has been defined by serological methods (6–10). Our results suggest that human T cells can be distinguished by virtue of the presence of a new antigenic marker, i.e., T-MICG. Furthermore, N cells, defined as E−, Slg−, can also be characterized by virtue of another protein, N-MICG. Although a fraction of N cells in man display the B-cell antigen (equivalent to mouse Ia, immune response associated antigen), this antigen is also found on B lymphocytes (35–39). In contrast, N-MICG is not found on either T or B-cell populations.

Further studies are in progress in our laboratory to determine the biological significance of these proteins.

Summary

Although surface immunoglobulin characterizes B cells in man, there are few surface markers that distinguish T cells. We have described a new protein synthesized in human T cells, termed T-MICG. This protein is a macromolecule of 225,000 daltons, is insoluble in the cold, and migrates as a β-globulin on electrophoresis. Separation of human peripheral blood lymphocytes into T and B-cell populations by

---

2 S. P. Hauptman et al. Manuscript in preparation.
rosette sedimentation and anti-human-Fab columns clearly demonstrated the T-cell origin of the 225,000 dalton component. Furthermore, null cells were shown to synthesize a protein of 185,000 daltons, termed N-MICG, with physical properties similar to T-MICG. T-MICG and N-MICG were shown to be antigenically dissimilar, employing antiserum to each of these proteins. The present studies demonstrate two novel cell surface markers, T-MICG and N-MICG, which characterize T cells and null cells, respectively.

Received for publication 14 August 1978.

References
1. Vitetta, E. S., Baur, S., and J. W. Uhr. 1971. Cell surface immunoglobulin. II. Isolation and characterization of immunoglobulin from mouse splenic lymphocytes. J. Exp. Med. 134:242.
2. Marchalonis, J. J. 1975. Lymphocyte surface immunoglobulins. Science (Wash. D. C.). 190:20.
3. Marchalonis, J. J., Cone, R. E., and H. Von Boehmer. 1974. Surface immunoglobulins of peripheral thymus-derived lymphocytes. Immunochemistry. 11:271.
4. Vitetta, E. S., Uhr, J. W., and E. A. Boyse. 1973. Immunoglobulin synthesis and secretion by cells in the mouse thymus that do not bear θ antigen. Proc. Natl. Acad. Sci. U. S. A. 70:834.
5. Reif, A. E., and J. M. V. Allen. 1964. The AKR thymic antigen and its distribution in leukemias and nervous tissues. J. Exp. Med. 120:413.
6. Raff, M. C. 1969. θ Isoantigen as a marker of thymus-derived lymphocytes in mice. Nature (Lond.). 224:378.
7. Cantor, H., and E. A. Boyse. 1977. Lymphocytes as models for the study of mammalian cellular differentiation. ImmunoL Rev. 33:105.
8. Wybran, J., and H. H. Fudenberg. 1973. Thymus-derived rosette-forming cells. N. Engl. J. Med. 288:1072.
9. Greaves, M. F., and G. Brown. 1974. Purification of human T and B lymphocytes. J. Immunol. 112:420.
10. Touraine, J. L., Hadden, J. W., and R. A. Good. 1977. Sequential stages of human T lymphocyte differentiation. Proc. Natl. Acad. Sci. U. S. A. 74:3414.
11. Hauptman, S. P., and G. Sobczak. 1978. A new protein: macromolecular insoluble cold globulin (MICG) derived from mouse thymocytes. Immunol. Commun. 7:69.
12. Hauptman, S. P. 1978. Macromolecular insoluble cold globulin (MICG): a novel protein from mouse lymphocytes I. Isolation and characterization. Immunochemistry. 15:415.
13. Hauptman, S. P., Sobczak, G., and I. A. Guterman. Macromolecular insoluble cold globulin (MICG): a novel protein from mouse lymphocytes. II. T-cell origin of MICG and response to mitogens. Immunochemistry. 15:423.
14. Boyum, A. 1967. Isolation of mononuclear cells and granulocytes from human blood. J. Clin. Lab. Invest. 21(Suppl. 97):77.
15. Alderson, E., Birchall, J. P., and J. J. T. Owen. 1976. A simple method of lymphocyte purification from human peripheral blood. J. Immunol. Methods. 11:297.
16. Aiuti, F., Cerottini, J. C., Coombs, R. A., Cooper, M., and H. B. Dickler et al. 1974. Identification, enumeration, and isolation of B and T lymphocytes from human peripheral blood. Scand. J. Immunol. 3:521.
17. Higmans, W. H., Schuit, H. R. E., and F. Klein. 1969. An immunofluorescence procedure for the detection of intracellular immunoglobulins. Clin. Exp. Immunol. 4:457.
18. Bianco, C., Patrick, R., and V. Nussenzweig. 1970. A population of lymphocytes bearing a membrane receptor for antigen-antibody-complement complexes. I. Separation and
characterization. J. Exp. Med. 132:702.

19. Chess, L., MacDermott, R. P., and S. F. Schlossman. 1974. Immunologic functions of isolated human lymphocyte subpopulations. 1. Quantitative isolation of human T and B cells and response to mitogens. J. Immunol. 113:1113.

20. Chess, L., MacDermott, R. P., Sondel, P. M., and S. F. Schlossman. 1974. Isolation and characterization of cells involved in human cellular hypersensitivity. Prog. Immunol. 3:125.

21. Chess, L., Levine, H., MacDermott, R. P., and S. F. Schlossman. 1975. Immunologic functions of isolated human lymphocyte subpopulations. VI. Further characterization of the surface Ig negative, E rosette negative (null cell) subset. J. Immunol. 115:1483.

22. Laskov, R., and M. D. Scharff. 1970. Synthesis, assembly and secretion of gamma globulin by mouse myeloma cells. I. Adaptation of the Merwin plasma cell tumor—11 to culture, cloning, and characterization of gamma globulin subunits. J. Exp. Med. 131:1515.

23. Laskov, R., Lanzerotti, R., and M.D. Scharff. 1971. Synthesis, assembly and secretion of gamma globulin by mouse myeloma cells. II. Assembly of IgGk immunoglobulin by MPC II tumor and culture cells. J. Mol. Biol. 56:327.

24. Hauptman, S. P., and T. B. Tomasi. 1974. A monoclonal IgM protein with antibody-like activity for human albumin. J. Clin. Invest. 53:932.

25. Hauptman, S. P., and T. B. Tomasi. 1974. Antibodies to human albumin in cirrhotic sera. J. Clin. Invest. 54:122.

26. Greenwood, F. C., and W. M. Hunter. 1963. The preparation of 131I-labeled human growth hormone of high specific radioactivity. Biochem. J. 89:114.

27. McConahey, P. J., and F. J. Dixon. 1966. A method of trace iodination of proteins for immunological studies. Int. Arch. Allergy Appl. Immunol. 19:185.

28. Weber, K., and M. Osborn. 1969. The reliability of molecular weight determinations by dodecyl sulfate-polyacrylamide gel electrophoresis. J. Biol. Chem. 244:4406.

29. Terasaki, P. I., and J. D. McClelland. 1964. Micro-droplet assay of human serum cytotoxins. Nature (Lond.). 204:998.

30. Mittal, K. K., Mickey, M. R., Singal, D. P., and P. I. Terasaki. 1968. Serotyping for homotransplantation. XVIII. Refinement of microdroplet lymphocyte cytotoxicity test. Transplantation (Baltimore). 6:913.

31. Pernis, B., Forni, L., and L. Amante. 1970. Immunoglobulin spots on the surface of rabbit lymphocytes. J. Exp. Med. 132:1001.

32. Higmans, W. H., Schult, H. R. E., and F. Klein. 1969. An immunofluorescence procedure for the detection of intracellular immunoglobulins. Clin. Exp. Immunol. 4:457.

33. Gooding, J.W. 1976. Conjugation of antibodies with fluorochromes: modifications to the standard methods. J. Immunol. Methods. 13:213.

34. Preud'Homme, J. L., and M. Seligmann. 1972. Anti-human immunoglobulin G activity of membrane-bound monoclonal immunoglobulin M in lymphoproliferative disorders. Proc. Natl. Acad. Sci. U. S. A. 69:2132.

35. Chess, L., Evans, R., Humphreys, R. E., Strominger, J. L., and S. F. Schlossman. 1976. Inhibition of antibody-dependent cellular cytotoxicity and immunoglobulin synthesis by an antisera prepared against a human B-cell Ia-like molecule. J. Exp. Med. 144:113.

36. Klareskog, L., Sanberg-Tragardh, L., Rask, L., Lindblom, J. B., Curman, B. and P. A. Peterson. 1977. Chemical properties of human Ia antigens. Nature (Lond.). 265:248.

37. Billing, R., Rafizadeh, B., Drew, I., Hartman, G., Gale, R., and P. Terasaki. 1976. Human B-lymphocyte antigens expressed by lymphocytic and myelocytic leukemia cells. 1. Detection by rabbit antisera. J. Exp. Med. 144:167.

38. Schlossman, S. F., Chess, L., Humphreys, R. E., and J. L. Strominger. 1976. Distribution of Ia-like molecules on the surface of normal and leukemic human cells. Proc. Natl. Acad. Sci. U. S. A. 73:1288.

39. Winchester, R., Wang, C. Y., Halper, J., and T. Hoffman. 1976. Studies with B-cell Allo- and Hetero-antisera: parallel reactivity and special properties. Scand. J. Immunol. 5:745.