Proc. Natl. Acad. Sci. USA
Vol. 77, No. 5, pp. 2914–2918, May 1980
Immunology

Characterization of a monoclonal antibody that defines an immunoregulatory T cell subset for immunoglobulin synthesis in humans

(human T cell antigens/immunoregulation/B cells)

BARTON F. HAYNES*, DEAN L. MANN†, MARTIN E. HEMLER‡, JOYCE A. SCHROER*, JAMES H. SHELHAMER*, GEORGE S. EISENBARTH§, JACK L. STROMINGER‡, CHARLES A. THOMAS*, HOWARD S. MOSTOWSKI*, AND ANTHONY S. FAUCI*

*Laboratory of Clinical Investigation, National Institute of Allergy and Infectious Diseases, and the †Immunology Branch, National Cancer Institute, National Institutes of Health, Bethesda, Maryland 20205; ‡Sidney Farber Cancer Institute, Boston, Massachusetts 02115; and §Duke University School of Medicine, Durham, North Carolina 27710

Contributed by Jack Leonard Strominger, March 5, 1980

ABSTRACT **This study characterizes a monoclonal antibody (3A1), and partially characterizes the cell surface antigen and the functional peripheral blood T cell subset that it defines. The 3A1 antigen is present on the surface of several human T cell lines (HSB-2, CEM, MOLT-4, and others) in various amounts but is absent from the T cell line YT4E and all human B cell lines tested. Immunoprecipitation of an HSB-2 extract with 3A1 yielded one specific band with a molecular weight of approximately 40,000 in the presence of reducing agent. With directly fluoresceinated 3A1 antibody, fluorescence-activated cell sorter analysis showed that 85% of peripheral blood E-rosette-positive T cells were positive for the 3A1 antigen. After E-rosette-positive cells had been separated into $3A1^+$ and $3A1^-$ cell suspensions, the $3A1^+$ cells helped autologous peripheral blood B cell suspensions toward pokeweed mitogen-driven proliferation and intracytoplasmic Ig production, whereas $3A1^-$ T cells did not. Further, addition of $3A1^-$ cells from some but not all normal subjects to cocultures of $3A1^+$ cells and B cells actively suppressed intracytoplasmic Ig production. However, the $3A1^+$ T cell subset could be activated by concanavalin A to maximally suppress B cell Ig synthesis *in vitro*. Thus, the 3A1 antibody defines a major functional subset of peripheral blood T cells and should provide a useful marker for the study of human T cell function.**

The regulation of human B cell Ig synthesis by thymus-derived (T) cells and soluble T cell factors has been well documented (1–5). The concept that lymphocyte differentiation antigens exist and code for various immunoregulatory functions has been given support by the demonstration of the functional correlates in humans of the Lyt series of T lymphocyte differentiation antigens in mice (6–10) and by studies, using heteroantisera against T cell subsets, suggesting an analogous series of human T cell differentiation antigens (11–15). Recently, by using the technique of Köhler and Milstein (16) for the production of myeloma lymphocyte hybrid cell lines, monoclonal antibodies reactive with a variety of lymphocyte cell surface differentiation antigens have been produced (17–19). In particular, we (20, 21) and others (22–25) have described monoclonal antibodies that react with various subsets of human peripheral blood (PB) T lymphocytes. We have described (20) the 3A1 antibody, which reacts with 80% of sheep erythrocyte (E)-rosette-positive T cells in PB. The present study reports purification of the 3A1 antibody and partial characterization of both the 3A1 antibody and antigen. We also describe the regulatory effect on Ig synthesis of the PB E-rosette-positive lymphocyte subset that the 3A1 antibody defines.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U. S. C. §1734 solely to indicate this fact.

MATERIALS AND METHODS

Production of Monoclonal Antibodies. 3A1 antibody was produced by a stable cloned murine lymphocyte hybrid cell line (20). The 3A1 cell line was established by fusion of BALB/c spleen cells from animals immunized with the HSB-2 cell line (a human T cell line) with the P3 × 63/Ag8 BALB/c myeloma cell line by the method of Galfre *et al.* (26).

Assay to Measure Cell Surface Antibody Binding. ^{125}I-labeled affinity-purified $F(ab)_2$ anti-mouse IgG antibodies were used to detect the specific binding of 3A1 antibody to cell surfaces as described (20, 21).

Isoelectric Focusing (IEF) Techniques. Preparative IEF was performed with a 440-ml column (LKB model 8100). A pH gradient of 5 to 8 was constructed in a sucrose gradient (50–0% wt/vol) in 3 M urea/1% ampholytes. The sample (4 ml of ascites fluid, specific antibody 10 mg/ml, in 3 M urea/25% sucrose) was applied to the middle of the gradient. The column was run at a constant 300 V for 72 hr at 4°C and eluted in 2-ml fractions. The anodal solution was 0.85% phosphoric acid and the cathodal solution was 1.6% (wt/vol) ethanolamine. Eluted fractions were dialyzed against phosphate-buffered saline at pH 7.4, concentrated by negative pressure dialysis, and either directly labeled with fluorescein isothiocyanate or isotyped by immunoelectrophoresis using affinity-purified goat anti-mouse Ig subclass reagents.

Analytical IEF of ascites containing murine monoclonal antibodies or their preparative IEF fractions was performed in a polyacrylamide slab gel system as described (27).

Cell Lines. The cell line YT4E was obtained from the laboratory of Ishii *et al.* (28); U-937 (29), from K. Nilsson; MANN, from the Genetics Laboratory, Oxford, and McBride B was a normal (transformed by Epstein–Barr virus) B cell line established in this laboratory. All other cell lines were obtained from either J. Minowada or H. Lazarus. The cell lines were maintained in RPMI 1640 medium supplemented with 10% fetal bovine serum (Microbiological Associates, Walkersville, MD) and grown at 37°C in a humidified atmosphere with 5% CO_2.

Characterization and Isolation of the 3A1 Antigen. HSB-2 T cells were treated with trypsin (type III, Sigma) at 5 mg/ml, Pronase (type IV, Sigma) at 600 μg/ml, or neuraminidase (*Clostridium perfringens*, type IV, Sigma) at 10 units/ml for

Abbreviations: Con A, concanavalin A; FACS, fluorescence-activated cell sorter; ICP, intracytoplasmic; IEF, isoelectric focusing; PB, peripheral blood; PWM, pokeweed mitogen; T, thymus-derived; E-rosette, rosette with sheep erythrocytes; NP-40, Nonidet P-40; SaCI, *Staphylococcus aureus* Cowan I strain.

45 min at 37°C. The cells were washed three times in RPMI 1640 medium supplemented with 20% fetal calf serum (GIBCO) and then assayed for their ability to bind 3A1 antibody in the ^{125}I-labeled anti-mouse IgG radioassay (20, 21). IODO-GEN (Pierce) was used to label the external proteins of cells with ^{125}I (30). Labeled cells were washed four times with RPMI 1640 medium, then extracted with 1% Nonidet P-40 (NP-40, Particle Data Laboratories, Elmhurst, IL) in 0.01 M Tris·HCl (pH 8.0)/0.5 M NaCl/2 mM phenylmethylsulfonyl fluoride (Sigma) for 1 hr at 0°C. After centrifugation for 10 min at 13,000 × *g*, the supernatant was used for immunoprecipitation (2 × 10^6 cpm per assay). *Staphylococcus aureus* Cowan I strain (SaCI) was used as an immunoadsorbent (31). The surface antigen immunoprecipitated with the 3A1 monoclonal antibody and SaCI was analyzed by $NaDodSO_4$/polyacrylamide gel electrophoresis (32).

Identification and Fractionation of PB Lymphocyte Subpopulations. Purified mononuclear cell suspensions were obtained from heparinized venous blood of 20 normal adults or from PB cell suspensions from leukapheresis of normal subjects (33). PB T cells were identified and separated by their ability to form rosettes spontaneously with sheep erythrocytes (34). This isolation method yielded E-rosette-positive suspensions containing >95% E-rosette-positive cells and E-rosette-depleted suspensions containing <5% E-rosette-positive cells. PB E-rosette-negative cell suspensions contained 45% surface-Ig-positive cells, 40% monocytes, and 15% non-E-rosetting, surface-Ig-negative lymphocytes. $3A1^+$ and $3A1^-$ cell suspensions were obtained by rosetting 3A1-coated T cells with staphylococcal protein A-coated bovine erythrocytes followed by centrifugation through a Ficoll/Hypaque gradient as described (20). Alternatively, purified T cells were separated into $3A1^+$ and $3A1^-$ subpopulations on the basis of adherence to 3A1-coated polystyrene petri dishes (100 × 15 mm) in a modification of the procedure of Mage *et al.* (35). With either method of separation, $3A1^+$ cell suspensions were ≧95% pure, while $3A1^-$ cell suspensions ≧90% pure.

Fluorescence-Activated Cell Sorter (FACS) Analysis. About 10^6 E-rosette-positive PB lymphocytes were incubated with a saturating amount of fluorescein conjugated 3A1 antibody (fluorescein-to-protein mole ratio, 2.0) at 20°C for 1 hr and washed twice in RPMI 1640 medium supplemented with 20% fetal calf serum. Flow microfluorometry was performed by using a FACS-II (Becton-Dickinson, Mountain View, CA) (36).

Functional Assays of Ig Synthesis. The ability of $3A1^+$ and $3A1^-$ T cells to induce and regulate pokeweed mitogen (PWM)-stimulated intracycloplasmic (ICP) Ig production by human B cells was assayed as described (5, 37). Culture and assay for PB concanavalin A (Con A) suppressor cell activity were performed as described (38, 39).

RESULTS

Characterization of the 3A1 Antibody. Preparative IEF was used to separate parent myeloma protein from lymphocyte hybrid myeloma protein that bound to HSB-2 cells (Fig. 1 *Upper*). Fig. 1 *Lower* shows the polyacrylamide gel IEF pattern of various fractions of the preparative IEF column in Fig. 1 *Upper*, as well as the patterns of parent P3 × 63/Ag8 (P3) ascites fluid and whole 3A1 ascites fluid. As can be seen, fraction D contained primarily parent P3 × 63/Ag8 monoclonal protein and contained little binding activity to HSB-2 cells, whereas fractions B and C contained no detectable P3 × 63/Ag8 Ig and contained most of the binding activity to HSB-2 cells from the preparative IEF column. Parent P3 × 63/Ag8 myeloma protein (fraction D) was isotyped as IgG_1, κ [as reported (40)], as were

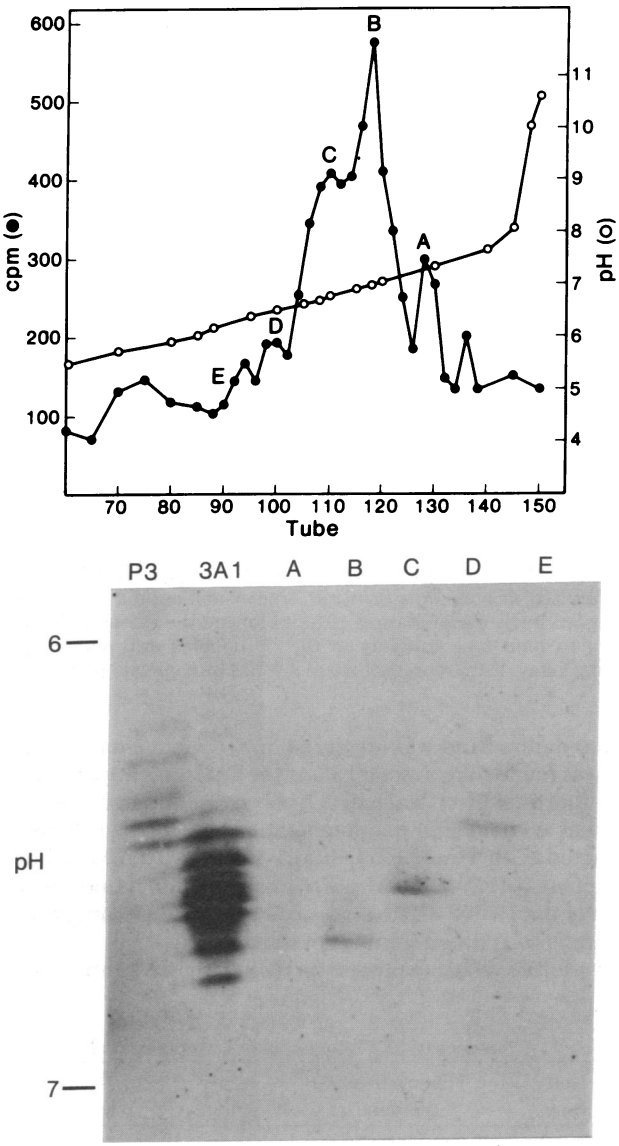

FIG. 1. IEF of 3A1 antibody. (*Upper*) 3A1 antibody was placed at the midpoint of a 50–0% wt/vol sucrose gradient in 3 M urea/1% ampholytes (pH 5–8) in a 440-ml IEF column. Eluted and dialyzed fractions were assayed for binding activity to HSB cells in the ^{125}I-labeled anti-mouse IgG binding assay (10,000 cpm total added). (*Lower*) Fractions were taken from points A–E and analyzed by IEF on a polyacrylamide gel with ampholytes (pH 5–8) and compared to the whole 3A1 myeloma protein (3A1) and parent P3 × 63/Ag8 myeloma protein (P3). P3 myeloma protein has a different spectrotype than 3A1 and, by using preparative IEF, parent P3 myeloma protein in 3A1 ascites (fraction D) was separated from 3A1 lymphocyte hybrid myeloma protein that had binding activity for HSB-2 cells (fractions C and B).

fractions B and C, which had high binding activity to HSB-2 cells.

Characterization of the 3A1 Antigen. We found that the 3A1 antigen on HSB cells was resistant to digestion with neuraminidase and highly sensitive to digestion with Pronase or trypsin (Fig. 2).

Immunoprecipitation of an ^{125}I-labeled extract of HSB-2 cells with 3A1 ascites fluid yielded a polypeptide of approximately 40,000 daltons as detected by electrophoresis in the presence of 2-mercaptoethanol (Fig. 3, lane C). This protein was not present on an SB cell extract (lane B) and was not precipitated by P3 × 63/Ag8 ascites fluid (lane D). The radioactivity in the

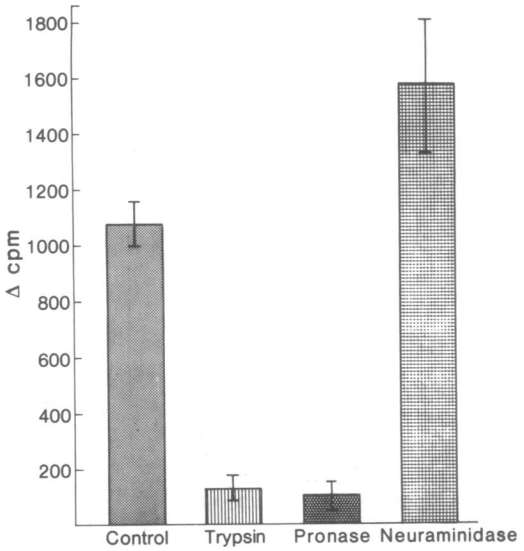

FIG. 2. Enzyme digestion studies of 3A1 antigen. HSB-2 T cells were treated with medium (control), trypsin at 5 mg/ml, Pronase at 600 μg/ml, or neuraminidase at 10 units/ml and then assayed for the ability to bind 3A1 antibody in the ^{125}I-labeled anti-mouse IgG binding assay. Data represent mean ± SEM of triplicate points.

40,000-dalton band was measured directly (cut from the gel) and was found to represent 0.3% of the total radioactivity in an HSB-2 extract after NaDodSO$_4$/polyacrylamide gel electrophoresis without immunoprecipitation. If HSB-2 cells were extracted with 1 mM EDTA/0.15 M NaCl/10 mM sodium phosphate (pH 7.4)/2 mM phenylmethylsulfonyl fluoride instead of the 1% NP-40 solution, no detectable 3A1 antigen was immunoprecipitated (results not shown).

In addition to being present on HSB-2, the 3A1 antigen was also present on several other human T cell lines (Table 1). HPB-MLT, MOLT-4, CEM, and TALL-1 showed reactivity comparable to that of HSB-2, whereas 3A1 binding was substantially less for RPMI-8402 and HPB-ALL and absent (near background) for YT4E. Human B cell lines (SB, MANN, and McBride B), a macrophage cell line (U-937), and a line from a myelogenous leukemia patient (K-562) also showed negligible reactivity.

Table 1. Presence of 3A1 antigen on hematopoietic cell lines*

Cell type	Hematopoietic cell line	Origin†	^{125}I bound, cpm (*n*)
T	HPB-MLT	LML	71,500 (4)
	HSB-2	T-ALL	61,000 (4)
	TALL-1	LML	49,900 (4)
	MOLT-4	T-ALL	45,000 (4)
	CEM	T-ALL	42,600 (4)
Less reactive T	RPMI-8402	T-ALL	25,300 (4)
	HPB-ALL	T-ALL	18,300 (4)
	YT4E‡	Sezary	2,040 (4)
B	SB	Normal§	1,890 (4)
	MANN	Normal§	680 (2)
	McBride B	Normal§	670 (2)
Other	K-562	CML-BC	3,760 (2)
	U-937	HL	3,130 (2)

* The results of cell surface binding assays for each cell line are given as the mean of the total number of experiments indicated in parentheses. Background binding (100–1200 cpm, mean 400 cpm ^{125}I) was determined for each cell line in the absence of monoclonal antibody and was <0.5% of the total cpm added (250,000 cpm). This has been subtracted to give the values shown. Control experiments with P3 ascites fluid resulted in background binding levels similar to those in experiments with no added monoclonal antibodies.

† LML, leukemic conversion of the malignant lymphoma; T-ALL, acute lymphoblastic leukemia with T cell membrane phenotype; CML-BC, chronic myelogenous leukemia in blastic crisis; HL, hystiocytic lymphoma (29).

‡ A hypotetraploid T cell line established by fusion of Sezary peripheral T cells (28).

§ Normal B cell lines transformed by Epstein–Barr virus.

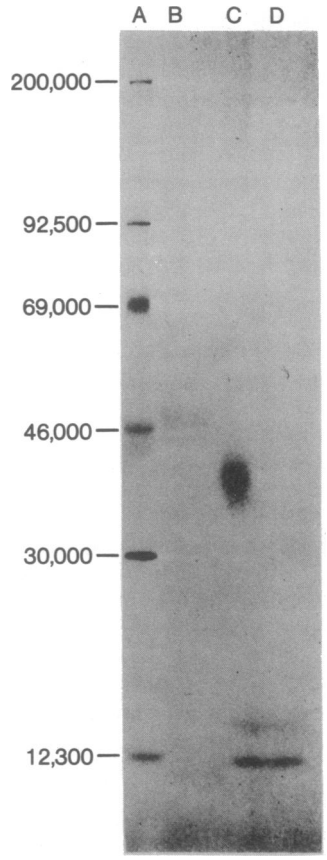

FIG. 3. Immunoprecipitation of the 3A1 antigen. Radiolabeled extracts of HSB-2 cell membranes were precleared twice, each time by incubation with normal mouse serum (20 min at 25°C) then SaCI (30 min at 4°C). After removal of the immunoadsorbent, the cleared extract was incubated with 0.25–1 μl of monoclonal antibody (ascites fluid) for 20 min at 25°C, and then with SaCI for 30 min at 4°C. The immunoadsorbent was then washed successively (at 0–4°C) with 1% NP-40/10 mM Tris·HCl (pH 8.0)/0.5 M LiCl (three times); 0.1% NP-40/10 mM Tris·HCl (pH 8.0) (two times); and 10 mM Tris·HCl (pH 8.0) (two times). The washed immunoadsorbent was boiled for 5 min in 0.1 M Tris·HCl (pH 6.8)/15% (vol/vol) glycerol/2% NaDodSO$_4$/2.5% 2-mercaptoethanol and analyzed by NaDodSO$_4$/polyacrylamide gel electrophoresis. The gels were composed of a linear gradient of 7–15% acrylamide. Analysis in the presence of 2-mercaptoethanol showed: protein standards (from the top) myosin, phosphorylase *a*, bovine serum albumin, ovalbumin, carbonic anhydrase, and cytochrome *c* (lane A); 3A1 immunoprecipitation of an SB cell extract (lane B); 3A1 immunoprecipitation of HSB-2 (lane C); P3 ascites fluid immunoprecipitation of HSB-2 (lane D).

Immunoprecipitation of ^{125}I-labeled extracts of HPB-MLT and MOLT-4 each yielded a single specific polypeptide of approximately 40,000 daltons, similar to that shown in Fig. 3.

FACS Analysis of Human PB E-Rosette-Positive Cell Suspensions. Fractions B and C from the preparative IEF column were combined and the purified 3A1 paraprotein was directly conjugated to fluorescein. When a saturating amount of 3A1 antibody was used, a mean of 85 ± 1% (SEM) of PB T cells were 3A1$^+$, while 15 ± 1% (SEM) were 3A1$^-$ in studies of

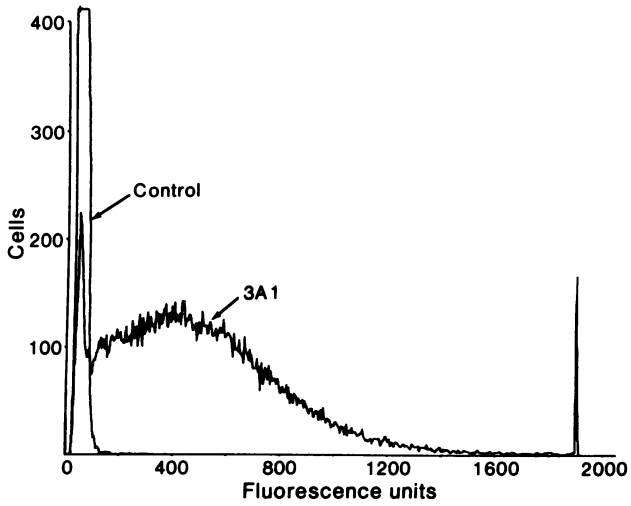

FIG. 4. FACS histogram of 3A1-labeled purified T cells from PB. A saturating amount of directly labeled IEF-purified 3A1 antibody was incubated with PB T cells, the cells were washed, and FACS analysis was performed. Control curve represents T cells incubated with directly fluoresceinated P3 × 63/Ag8 IEF-purified myeloma protein. In this figure 90% of T cells are $3A1^+$ while 10% are $3A1^-$.

18 normal donors (Fig. 4). Similar studies showed no specific binding of 3A1 antibody to purified PB monocytes, B cells, polymorphonuclear cells, or erythrocytes (data not shown).

Functional Studies of Regulation of Ig Synthesis. Total E-rosette-positive T cells (sham separated after treatment with 3A1 antibody) when added to PB B cell suspensions induced

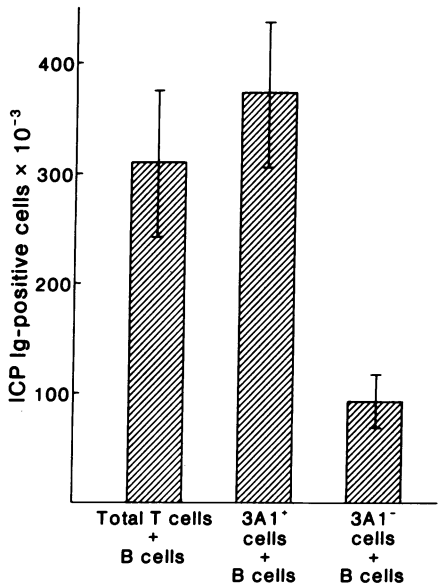

FIG. 5. $3A1^+$ and $3A1^-$ T cell subsets as inducers of Ig synthesis *in vitro*. Suspensions of 0.25 × 10^6 E-rosette-positive (total T) cells, $3A1^+$ cells, or $3A1^-$ cells were added to suspensions of 0.25 × 10^6 T-cell-depleted (B cell) PB mononuclear cells, and their ability to induce ICP Ig in the presence of PWM (1:200 final dilution, GIBCO) was assayed. Cells were cultured in 1 ml total volume of RPMI 1640 medium supplemented with 10% human type A serum in 5% CO_2 in air, 37°C, 100% humidity on a rocker platform. At the end of 6 days in culture the cells were harvested and washed, and cell yield and viability were determined. A cytopreparation was made and, after fixation in 5% acetone/95% ethanol (vol/vol) at −20°C, stained with fluoresceinated rabbit anti-human polyvalent Ig (Meloy Laboratories, Springfield, VA) (0.5 mg/ml) at 20°C for 45 min. After washing with cold phosphate-buffered saline, the slides were read on a Zeiss fluorescent microscope. Data represent the mean ± SEM of six experiments.

Table 2. Ability of $3A1^-$ cells to actively suppress PWM-stimulated ICP Ig synthesis

	ICP Ig-positive cells × 10^{-3}					
	$3A1^+$ cells added × 10^{-6}			$3A1^-$ cells added × 10^{-6}		
Exp.	0.25	0.5	1.0	0.25	0.5	1.0
1	487	737	468	160	253	34
2	498	573	653	425	284	242
3	210	240	100	270	30	60
4	245	305	225	535	390	300

Indicated numbers of $3A1^+$ or $3A1^-$ cells were added to 0.25 × 10^6 $3A1^+$ + 0.25 × 10^6 B cell cocultures. PWM-stimulated B cells in the absence of any added T cells contained fewer than 30 × 10^3 ICP-positive cells.

317,000 ± 67,000 (mean ± SEM) ICP Ig-positive cells per culture (Fig. 5). B cell suspensions alone as well as all T cell suspensions alone produced fewer than 30,000 ICP Ig-positive cells. Cocultures of $3A1^+$ cells + B cells contained 373,000 ± 67,000 ICP Ig-positive cells per culture. However, cocultures of $3A1^-$ cells + B cells contained only 97,000 ± 25,000 ICP Ig-positive cells ($P < 0.001$, compared to coculture with $3A1^+$). In addition, the yields of viable cells in cocultures of $3A1^+$ cells + B cells were identical to those of total T cell + B cell cocultures (1,268,000 ± 138,000 vs. 1,242,000 ± 123,000 cells) ($P > 0.2$). In contrast, the yield of viable cells in cocultures of $3A1^-$ cells + B cells was no different from the original number of cells placed in culture (718,000 ± 135,000 cells).

Next we determined the effect on PWM-driven Ig synthesis of adding graded numbers of total T cells, $3A1^+$ T cells, or $3A1^-$ T cells to B cell suspensions. Up to 1 × 10^6 $3A1^-$ cells added to B cells did not help toward Ig synthesis, whereas comparable numbers of total T or $3A1^+$ cells did help PWM-stimulated ICP Ig synthesis (data not shown).

Next, graded numbers of $3A1^+$ or $3A1^-$ cells were added to cultures of 0.25 × 10^6 $3A1^+$ cells + 0.25 × 10^6 B cells to see if $3A1^-$ cells could actively suppress Ig synthesis in a "helper" system of Ig production.

As can be seen in Table 2, in some experiments, the addition of graded numbers of $3A1^-$ cells to cocultures of $3A1^+$ cells + B cells suppressed the numbers of ICP Ig-positive cells to various degrees.

To determine if Con A could trigger $3A1^+$ cells to suppress Ig synthesis, $3A1^+$ cells were cultured in the presence (Con A-activated cells) or absence (control cells) of Con A at 10 μg/ml (38, 39). After 2 days, control or Con A-activated cells were added to PWM-stimulated PB mononuclear cell cultures in a 1:1 ratio. In four separate experiments we found that Con A-activated $3A1^+$ T cells suppressed PWM ICP Ig synthesis to 9.7 ± 9% of the responses seen when control cells were added ($P < 0.05$).

DISCUSSION

The present study has characterized the 3A1 antibody as a murine IgG_1, κ monoclonal myeloma protein. This antibody was separated from parent (P3 × 63/Ag8) myeloma protein by preparative IEF techniques. The previously described (20) T cell specificity of the 3A1 antibody has been reaffirmed for both human PB T cells and T cell lines. As measured by FACS analysis, the 3A1 antibody bound to a majority (85%) of human PB E-rosette-positive cells. This is consistent with the finding that only one T cell line (YT4E) out of the eight lines tested was negative for 3A1 binding. However, the meaning of apparently diminished expression on Sezary T cell lines awaits further investigation.

The antigenic determinants to which 3A1 antibody binds were digested by tryspin and Pronase but were resistant to

neuraminidase treatment. The 3A1 antigen appears to be a single polypeptide with an approximate molecular weight of 40,000; it is probably an integral membrane protein, because detergent was needed to solubilize it. Assuming average labeling and quantitative immunoprecipitation, the percentage (0.3%) of the total membrane radioactivity corresponding to the 3A1 antigen may approximate the relative amount of this membrane component.

Functionally, $3A1^+$ cells help PWM-driven ICP Ig production, whereas the $3A1^-$ cells do not. Moreover, in some experiments $3A1^-$ cells were suppressors of ICP Ig production in cocultures of $3A1^+$ cells + B cells.

Jandinski *et al.* (8) showed that murine Lyl T cells after Con A activation helped B cells toward Ig synthesis, whereas Con A-activated Ly2,3 T cells suppressed B cell Ig synthesis. These data suggested that *in vitro* helper and suppressor T cell functions were manifestations of separate T cell differentiation pathways (8). Whereas only $3A1^+$ T cells helped B cells toward PWM-induced Ig synthesis, $3A1^+$ T cells were activated by Con A to suppress Ig synthesis to the same degree (10% of control responses) as do Con A-activated total T cells (38, 39). Thus, regarding Ig synthesis and Con A-inducible suppressor T cells, 3A1 antibody does not define a human T cell subset analogous to the murine Lyl subset.

Reinherz and colleagues (24, 25) have described the OKT4 antibody, which defines the human helper T cell for induction of Ig synthesis and for the generation of cytotoxic T cells in mixed lymphocyte cultures. Fifty-five percent of human PB T cells are $OKT4^+$, whereas in comparison 85% are $3A1^+$. In addition, $OKT4^+$ T cells proliferate normally in response to Con A and to phytohemagglutinin (24). $3A1^+$ E-rosette-positive cells proliferate normally in response to both phytohemagglutinin and Con A (20). Thus, $OKT4^+$ T cells may be a subset of $3A1^+$ T cells. The 3A1 antibody also appears to be different from the OKT1 antibody described by Reinherz *et al.* (23) in that all human PB T cells react with OKT1 whereas only 85% react with 3A1.

Thus, we have characterized the 3A1 antibody, which defines a major functional human T cell subset containing the human helper cell for Ig synthesis. This reagent should prove extremely useful in the study of human T lymphocyte function. The 3A1 antibody is available in limited quantities upon request.

1. Keightley, R. G., Cooper, M. D. & Lawton, A. R. (1976) *J. Immunol.* **117,** 1538–1544.
2. Waldmann, T., Broder, S., Blaese, R. M., Durm, M., Blackman, M. & Strober, W. (1974) *Lancet* **ii,** 609–613.
3. Moretta, L., Webb, S. R., Grossi, C. H., Lydyard, P. M. & Cooper, M. D. (1977) *J. Exp. Med.* **146,** 184–200.
4. Fauci, A. S., Pratt, K. R. & Whalen, G. (1976) *J. Immunol.* **117,** 2100–2104.
5. Haynes, B. F. & Fauci, A. S. (1979) *J. Immunol.* **123,** 1289–1298.
6. Cantor, H. & Boyse, E. A. (1975) *J. Exp. Med.* **141,** 1376–1389.
7. Cantor, H. & Boyse, E. A. (1975) *J. Exp. Med.* **141,** 1390–1399.
8. Jandinski, J., Cantor, H., Tadakuma, T., Peavy, D. L. & Pierce, C. W. (1976) *J. Exp. Med.* **143,** 1382–1390.
9. Eardley, D. D., Hugenberger, J., McVay-Bondreau, L., Shen, F. W., Gershon, B. K. & Cantor, H. (1978) *J. Exp. Med.* **147,** 1106–1115.
10. Cantor, H., McVay-Bondreau, L., Hugenberger, J., Naidorf, K., Shen, F. W. & Gershon, R. K. (1978) *J. Exp. Med.* **147,** 1116–1125.
11. Evans, R. L., Breard, J. M., Lazarus, H., Schlossman, S. F. & Chess, L. (1977) *J. Exp. Med.* **145,** 221–231.
12. Evans, R. L., Lazarus, H., Penta, A. C. & Schlossman, S. F. (1978) *J. Immunol.* **120,** 1423–1428.
13. Stevens, R. H. & Saxon, A. (1978) *Clin. Immunol. Immunopathol.* **10,** 438–445.
14. Reinherz, E. L. & Schlossman, S. F. (1979) *J. Immunol.* **122,** 1335–1341.
15. Pratt, D. M., Schlossman, S. F. & Strominger, J. L. (1980) *J. Immunol.* **124,** 1449–1461.
16. Köhler, G. & Milstein, C. (1976) *Eur. J. Immunol.* **6,** 511–519.
17. Melchers, F., Potter, M. & Warner, N. L. (1978) in *Lymphocyte Hybridomas,* eds. Melchers, F., Potter, M. & Warner, L. N. (Springer, New York), pp. 9–23.
18. Levy, R., Dilley, J. & Lampson, L. A. (1978) in *Lymphocyte Hybridomas,* eds. Melchers, F., Potter, M. & Warner, L. N. (Springer, New York), pp. 164–169.
19. Trucco, M. M., Stocker, J. W. & Ceppellini, R. (1978) *Nature (London)* **273,** 666–668.
20. Haynes, B. F., Eisenbarth, G. S. & Fauci, A. S. (1979) *Proc. Natl. Acad. Sci. USA* **76,** 5829–5833.
21. Eisenbarth, G. S., Haynes, B. F., Schroer, J. A. & Fauci, A. S. (1980) *J. Immunol.* **124,** 1237–1244.
22. Kung, P. C., Goldstein, G., Reinherz, E. L. & Schlossman, S. F. (1979) *Science* **206,** 347–349.
23. Reinherz, E. L., Kung, P. C., Goldstein, G. & Schlossman, S. F. (1979) *J. Immunol.* **123,** 1312–1317.
24. Reinherz, E. L., Kung, P. C., Goldstein, G. & Schlossman, S. F. (1979) *Proc. Natl. Acad. Sci. USA* **76,** 4061–4065.
25. Reinherz, E. L., Kung, P. C., Goldstein, G. & Schlossman, S. F. (1979) *J. Immunol.* **123,** 2894–2896.
26. Galfre, G., Howe, S. C., Milstein, C., Butcher, G. W. & Howard, J. C. (1977) *Nature (London)* **266,** 550–552.
27. Briles, D. E. & Davie, J. M. (1975) *J. Immunol. Methods* **8,** 363–372.
28. Ishii, K., Yodoi, J., Hanaoka, M. & Furuyama, J. I. (1978) *J. Cell. Physiol.* **94,** 93–98.
29. Sundström, C. & Nilsson, K. (1976) *Int. J. Cancer* **17,** 565–577.
30. Markwell, M. A. K. & Fox, C. F. (1978) *Biochemistry* **17,** 4807–4817.
31. Kessler, S. W. (1975) *J. Immunol.* **115,** 1617–1624.
32. Laemmli, U. K. (1970) *Nature (London)* **227,** 680–685.
33. Haynes, B. F. & Fauci, A. S. (1978) *J. Immunol.* **121,** 559–565.
34. Jondal, M., Holm, G. & Wigzell, H. (1972) *J. Exp. Med.* **136,** 207–215.
35. Mage, M. C., McHugh, L. L. & Rothstein, T. L. (1977) *J. Immunol. Methods* **15,** 47–56.
36. Benna, W. A., Hulett, H. R., Sweet, R. G. & Herzenberg, L. A. (1972) *Rev. Sci. Instrum.* **43,** 404.
37. Kearney, J. F. & Lawton, A. R. (1975) *J. Immunol.* **115,** 671–676.
38. Haynes, B. F. & Fauci, A. S. (1977) *J. Immunol.* **118,** 2281–2287.
39. Haynes, B. F. & Fauci, A. S. (1978) *Cell. Immunol.* **36,** 294–302.
40. Cowan, N. J., Seeher, D. S. & Milstein, C. (1974) *J. Mol. Biol.* **90,** 691–701.