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Further Studies on Th-B, A Cell Surface Antigenic Determinant Present on Mouse B Cells, Plasma Cells and Immature Thymocytes[1]

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A goat antiserum (Goat anti-M104E) has been produced which contains antibodies selectively cytotoxic for mouse B cells and a subpopulation of thymus cells. It reacts with the Th-B antigenic determinant which has been shown by us (1–3) to be present on B cells and on plasma cells and on some cells in the thymus. It also is very cytotoxic for mouse B cells while a previously developed rabbit antiserum was not. The antiserum was obtained by immunization with cells of the BALB/c mouse myeloma MOPC-104E. When the antiserum was purified by *in vivo* absorption in mice, antibodies remained which were cytotoxic for cells of all of several myelomas at a titer between 1:128 and 1:1024 as determined by an *in vitro* complement dependent cytotoxicity test. The *in vivo* purified antibodies were also cytotoxic for about 70% of thymus cells, for about 70% of spleen cells, for about 50% of lymph node cells and for about 20% of bone marrow cells. They were very cytotoxic for splenic or lymph node B cells separated from T cells by a nylon wool column and only slightly cytotoxic for splenic or lymph node T cells. The antibodies were only weakly cytotoxic for one out of five T cell tumors tested and not cytotoxic for the remaining four. Irrespective of target cells used, the cytotoxicity of purified Goat anti-M104E was easily removed by absorption with cell suspensions from tissues which contain B cells, plasma cells or thymus cells. In order to confirm that the same anti-Th-B antibodies recognize the determinant present on spleen cells and on some thymocytes, the purified Goat anti-M104E serum was absorbed with either spleen cells or thymus cells. The absorbed sera were tested for ability to label thymocytes or spleen cells using the fluorescence activated cell sorter (FACS). Either absorption removed essentially all the antibody capable of binding to either cell population. In addition it was shown, using the FACS, that only B cells and not T cells of the spleen contain the Th-B determinant. The anti-Th-B antibodies have now been used for the rapid elimination of B cells from a mixed population of lymphocytes without affecting the function of mature T cells. Thus *in vitro* treatment of spleen cells from SRBC-immunized donors with purified Goat anti-M104E plus complement results in the killing of a high proportion of the B memory cells as shown by the reduction of PFC produced when the treated cells

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are transferred to irradiated recipients. The T cell helper function of the transferred cells is not affected by Goat anti-M104E treatment as shown by appropriate cell transfer experiments in which effective B cells are provided by an AKR anti-Thy-1.2-treated spleen cell population and effective T cells are provided by the Goat anti-M104E-treated spleen cell population. Antibodies detecting Th-B may serve as an approach to understanding the ontogeny of lymphocytes. Our results suggest that Th-B is a cell surface marker appearing early in the development of lymphoid cells, on the common precursor of B and T cells and that it is lost from T cells as they mature in the thymus.

INTRODUCTION

Much work has been reported on the detection of cell surface markers on lymphoid cells. Among the markers described for mouse bone marrow-derived lymphocytes (B cells) which are precursors of antibody forming cells are the following: the immunoglobulin present in high density on the cell surface (4–6), the receptors for antigen-antibody complexes (7), for aggregated IgG (8) and for activated C3 (9), and the other membrane antigens such as MBLA (10) and Ly 4 (11). Another surface alloantigen PC-1 (12) and a xenoantigen which we have detected by rabbit antisera raised against BALB/c mouse IgG- or IgA-producing myeloma cells (13) seem to be more selectively expressed on plasma cells. More recently it has been claimed that thymus-derived lymphocytes (T cells) have the same or similar markers. For example, they also bear Fc receptors for antigen-antibody complexes (14) and aggregated IgG (15). Furthermore, the presence of immunoglobulin on the surface of thymus cells and T cells has been repeatedly reported by different investigators (16–19) and, recently, active synthesis of immunoglobulin receptors for antigen by T cells has been clearly shown by Roelants *et al.* (20). These various properties previously thought to be limited to B cells and now found on T cells, however, appear to be relatively less expressed on T cells than on B cells. One hypothesis suggested by these findings, together with the observations that even T cells probably originate in the bone marrow in adults (21–23), is that there are stem cells common to B and T pathways and that at least some T cells, or more particularly, their predecessors, still retain some of the properties common to B cells.

The Th-B marker which we described previously emphasizes this relationship since it is a surface antigenic determinant shared by B cells and a subpopulation of thymus cells but not by mature T cells. This determinant was previously detected by rabbit antiserum against cells of the BALB/c mouse myeloma MOPC-104E. We have now found antibodies to this determinant in a goat antiserum (Goat anti-M104E) also raised against cells of the BALB/c mouse IgM myeloma MOPC-104E and purified by *in vivo* absorption in mice. The purified Goat anti-M104E differed from the rabbit antiserum in that the goat antiserum was found to be selectively cytotoxic for splenic and lymph node mouse B cells whereas the rabbit antiserum was not. Both antisera were cytotoxic for normal and malignant plasma cells and a major subpopulation of normal thymus cells. Neither antiserum was cytotoxic for mature T cells. Furthermore, fluorescent labeled antibody studies, utilizing the fluorescence activated cell sorter (FACS), have shown that the labeled antibodies which combine with the Th-B determinant are effectively removed by adsorption with either thymus or spleen cells. In addition, fractionation of spleen cells labeled with antibody against brain associated T(BAT) antigen on the FACS has shown that the BAT negative spleen cells, i.e., splenic B cells, are the ones bearing Th-B.

TABLE 1
Identification of the Myelomas and Other Tumors Tested for Sensitivity to the Cytotoxic Action of Purified Goat Anti-M104E

Designation	Mouse strain of origin	Type	Immunoglobulin product	Carried as ascites or subcutaneous transplant	Cytotoxic titer of purified Goat anti-M104E[a]
MOPC-104E	BALB/c	Myeloma	IgM and λ-type Bence Jones protein	Subcutaneous	1024
TEPC-183	BALB/c	Myeloma	IgM	Subcutaneous	512
McPC-774	BALB/c	Myeloma	IgM	Subcutaneous	512
MOPC-21	BALB/c	Myeloma	Non-producer[b]	Ascites	128
ADJ-PC-5	BALB/c	Myeloma	IgG_{2a}	Subcutaneous	128
TEPC-15	BALB/c	Myeloma	IgA	Ascites	128
MPC-1	BALB/c	Myeloma	IgA	Ascites	256
HOPC-8	BALB/c	Myeloma	IgA	Subcutaneous	256
MOPC-315-Op	BALB/c	Myeloma	IgA	Subcutaneous	128
X5563	C3H/He	Myeloma	IgG_{2a}	Subcutaneous	128
BUGSY 40	BALB/c	Lymphoma	—	Ascites	8
P1798	BALB/c	Lymphoma	—	Subcutaneous	0[c]
PU-5(#16)	BALB/c	Lymphoma	—	Ascites	0
6C3HED	C3H/He	Lymphoma	—	Ascites	0
EL-4	C57BL	Lymphoma	—	Ascites	0
L1210	DBA/2	Leukemia	—	Ascites	0
Ehrlich[d]	Unknown	Undifferentiated	—	Ascites	0

[a] Reciprocal of highest antiserum dilution which kills 50% of the total target cells.
[b] MOPC-21 has been producing IgG_1, but changed into non-paraprotein producing myeloma during maintenance in our laboratory.
[c] Even neat antiserum did not show any significant cytotoxicity.
[d] Ehrlich tumor cells were carried in Swiss mice.

Our current studies also show that purified Goat anti-M104E can suppress the fuction of B cells without affecting the helper activity of T cells. Thus, this purified Goat anti-M104E is useful for rapid elimination of B cells from a mixed population of lymphocytes. In addition, the Th-B antigen, by which B cells and the subpopulation of thymus cells can be identified, may serve as an approach to understanding the ontogenic history of lymphocytes.

MATERIALS AND METHODS

Mice

Inbred mice strains BALB/c, DBA/2, C57BL/6J, AKR/Sn, B10.D2 and C3H/He and outbred Swiss mice 6 to 12 weeks old were used and were obtained either from the West Seneca Laboratories of Rosewell Park Memorial Institute or from the Jackson Laboratory. Nude mice (nu/nu) were obtained through the courtesy of Dr. B. A. Croy of the Hospital for Sick Children, Toronto, Canada.

Transplanted Tumors

Table 1 identifies the different lines of mouse myeloma and other tumors used in the present study. Among these tumors. P1798, EL-4, and 6C3HED were found

to possess Thy-1.2 antigen by their high sensitivity to anti-Thy-1.2 antiserum in a cytotoxicity test. BUGSY 40 is also Thy-1.2 positive although it is less sensitive to anti-Thy-1.2 antiserum than are the three T cell tumors described above. PU-5(#16) is also known to bear Thy-1 antigen by immunofluorescent technique (R. Asofsky, personal communication) although it failed to lyse completely with optimal concentration of anti-Thy-1 antiserum by cytotoxicity test. Myelomas are known to be negative for the Thy-1 antigen (12, 24).

Preparation of Goat Antiserum Against MOPC-104E Cells (Goat anti-M104E)

Antiserum against BALB/c mouse myeloma MOPC-104E cells (IgM and λ-type Bence Jones protein producer) was obtained by injection of a cell suspension of MOPC-104E cells (over 90% viable) prepared as described previously (25) into a goat. Cells (5×10^8) emulsified in Freund's complete adjuvant were injected intramuscularly into several sites followed by two injections of the same amount of cells in Freund's incomplete adjuvant at 3-week intervals. Starting 10 days after the last injection, the goat was bled three times at 2-week intervals and the sera obtained were combined to form one pool, which was heat inactivated for 30 min at 56°C and kept frozen until used.

Purification of Goat anti-M104E

Antiserum was purified by the combination of *in vitro* and *in vivo* absorption. It was first absorbed *in vitro* by being stirred for 60 min at 4°C with one-third volume of washed, packed Ehrlich tumor cells. This was repeated one more time. Portions, 0.8 ml of this *in vitro* preabsorbed Goat anti-M104E were injected ip into normal BALB/c mice. Fifteen hours after receiving the injection, the mice were bled from the retro-orbital sinus with a disposable pipette: to increase recovery of antibody, 1.5 ml saline was injected intravenously immediately before the mice were bled. The antiserum thus purified by *in vitro* and *in vivo* absorption is referred to as purified Goat anti-M104E. Normal goat serum (NGS) was also similarly purified.

Anti-Thy-1.2 Alloantiserum

Alloantiserum against Thy-1.2 antigen was prepared by a method similar to that described by Reif and Allen (26). AKR/Sn mice were injected four times at biweekly intervals with 10^7 C3H/He thymus cells. The cytotoxic titer of anti-Thy-1.2 serum against BALB/c thymus cells was 512 (the reciprocal of serum dilution killing 50% of the cells). Removal of anti-Thy-1 activity from the anti-Thy-1.2 serum was affected by absorptions with BALB/c brain cell suspensions. Absorptions with myeloma cells did not remove anti-Thy-1 preferentially.

Cell Suspensions

Single-cell suspensions from the tumors, spleen, mesenteric lymph node, thymus and bone marrow with high viability were prepared as described previously (13) in chilled Eagle's minimum essential medium (MEM).

Rabbit antiserum against mouse brain associated T antigen (Anti-BAT) was prepared by methods described elsewhere (27).

Cytotoxicity Test

A dye exclusion cytotoxicity test, which has been previously described in detail (28, 29), was employed. Briefly, to 0.1 ml serial dilutions of antiserum to be tested, 0.1 ml cells (5×10^6/ml in MEM) were added. Normal rabbit serum (0.1 ml), previously absorbed with myeloma, lymphoma and spleen cells and diluted 1:4, was added as a complement source. When thymus cells were tested, the complement was further absorbed with thymus cells before use. The mixture was gently shaken at 37°C for 45 min. The proportion of dead cells was determined by the addition of 0.1 ml 0.16% trypan blue. Antiserum alone or complement alone was used as a negative control.

Separation of Splenic T and B Cells on Nylon Wool

The method was based on that originally described in detail by Julius, Simpson and Herzenberg (30) and modified by Trizio and Cudkowicz (31). Effluent cells (non-adherent cells) and cells eluted from nylon wool (nylon wool adherent cells) were finally washed with and suspended in MEM. Spleen cells, thus separated, were usually more than 85% viable and were adjusted to a concentration of 5×10^6 viable cells/ml when used for cytotoxicity tests.

Assay for Plaque-Forming Cells (PFC Assay)

Detection of antigentic determinant on PFC (normal immunoglobulin secreting cells) was carried out as previously described (13). Namely, spleen cells obtained from BALB/c mice which had been immunized with sheep red blood cells (SRBC) were similarly treated *in vitro* as in the cytotoxicity test with complement plus purified Goat anti-M104E (diluted 1:50) which had not been absorbed or was further absorbed with various cells or with complement plus NGS before assay for PFC.

Absorption Test

The purified Goat anti-M104E was further absorbed *in vitro* with various mouse tissues or cells to determine whether an antigen detected by purified Goat anti-M104E was present or absent on the tissue to be tested. Based on the report of Takahashi *et al.* (12), a dilution two to three tubes below the cytotoxic end point of the purified Goat anti-M104E was selected for the absorption tests. The antiserum diluted to this point was incubated with an equal volume of washed homogenized tissue sediments, an equal volume of washed packed cells, or counted numbers of washed packed cells at 4°C for 60 min with periodic pipetting. The absorbed antiserum was recovered after centrifugation (8000*g* for 2 min, Brinkman micro-centerifuge 3200) and tested for its residual activity by PFC test or by cytotoxicity test against MOPC-104E cells, spleen cells or thymus cells.

Cell Transfer

Single-cell suspensions of spleen were obtained from BALB/c mice which had been immunized ip with 0.5 ml of 20% SRBC 4–6 weeks before. The treatment of the spleen cells was performed as follows: To 5×10^7 viable cells in 1.0 ml of MEM, were added 1.0 ml of a 1/4 dilution of purified Goat anti-M104E, purified

NGS, AKR anti-Thy-1.2 antiserum, or normal AKR mouse serum and 1.0 ml of a 1/4 dilution of rabbit serum. (complement). Following incubation at 37°C for 45 min, the cells were washed twice with cold MEM, suspended in an appropriate amount of MEM and injected iv in total doses of 1–3 × 10^7 cells per X-irradiated syngeneic recipient mouse. Irradiation of recipients was to a level of 600 R whole body, delivered 2–3 hr before cell transfer from a G.E. Maxitron 250 kV X-ray machine (240 kV at 30 mA; target distance 50 cm; added filtration was 0.25 Cu 1.0 Al; dose rate 120 R/min). In some cases, spleen cells treated with purified Goat anti-M104E were mixed with spleen cells treated with anti-Thy-1.2 antiserum and the mixture injected. About 1 hr later the recipients were injected iv with 0.2 ml of 20% SRBC. Five days after cell transfer, recipients were killed and spleens were individually analyzed for the presence of PFC.

Fluorescein Labeling and Cell Separation

Preparation of fluorescein labeled antibodies has been described previously (2). The staining of cells with fluorescent antibody and the separation and analysis of labeled cell populations on the FACS was carried out as reported (2). The fluorescein labeled antibody used to detect goat antibody which had reacted with cells was the Ig fraction of rabbit anti-goat Ig.

RESULTS

Cytotoxic Activity of Purified Goat anti-M104E

The specificity of purified Goat anti-M104E was tested by its cytotoxicity against various mouse tumor cells and normal BALB/c lymphoid cells. As shown in Fig. 1, reactions were positive for all myeloma cells tested to a titer between 1:128 and 1:1024, for about 70% of thymus cells, for about 70% of spleen cells, for about 50% of mesenteric lymph node cells and for about 20% of bone marrow cells. The antiserum was not cytotoxic for other tumor cells tested except BUGSY 40 cells which were killed at much lower titer (1:8) compared with myeloma cells (see

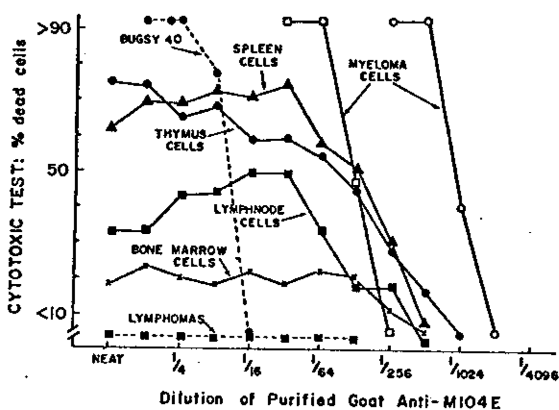

FIG. 1. Cytotoxic activity of purified Goat anti-M104E serum for various mouse tumor cells and normal BALB/c lymphoid cells. Note especially the strong cytotoxicity for myeloma cells, about 70% of spleen cells, about 60% of thymus cells, appreciable percentages of lymph node and bone marrow cells and absence of cytotoxicity for lymphoma cells.

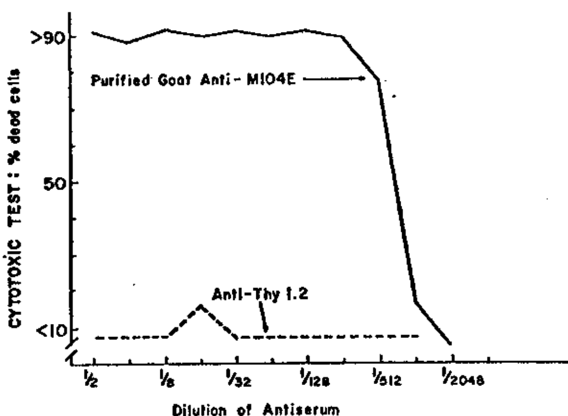

FIG. 2a. Cytotoxic activity of purified Goat anti-M104E serum for Thy 1.2 negative (B) spleen cells.

Table 1 also). Depending on the myeloma cells tested, their sensitivity to the killing effect of purified Goat anti-M104E differed. The homologous MOPC-104E cells were most efficiently killed; IgM-producing myelomas, McPC-774 and TEPC-183, were relatively sensitive compared with other myelomas which are producers of IgG or of IgA or non-paraprotein producers (Table 1). Cytotoxicity of purified Goat anti-M104E cannot be attributed to antibodies against mouse serum components because (a) purified Goat anti-M104E did not reveal any precipitin line by immunoelectrophoresis with normal BALB/c mouse serum or with serum from BALB/c mice bearing MOPC-104E, and (b) serum from BALB/c mice bearing MOPC-104E did not inhibit the cytotoxicity of purified Goat anti-M104E. Our purification method also completely eliminated antibodies against mouse and sheep RBC as shown by hemagglutination tests.

The cytotoxic activity of purified Goat anti-M104E against spleen cells or thymus cells was also tested against cells obtained from mice of various strains including AKR/Sn mice and C3H/He mice. For these strains, about 70–80% of spleen cells and about 60–70% of thymus cells were killed by the antiserum as were BALB/c mouse cells. A somewhat higher proportion (90%) of spleen cells from nude mice

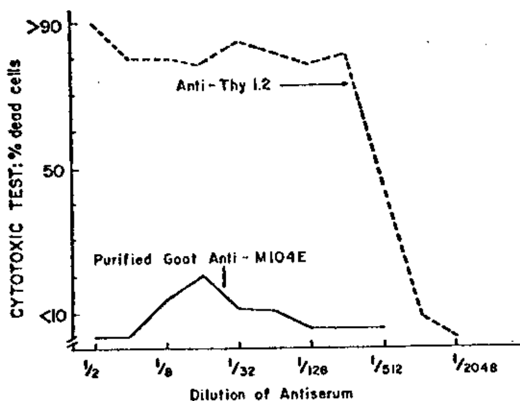

FIG. 2b. Absence of cytotoxic activity of purified Goat anti-M104E serum for Thy 1.2 positive (T) spleen cells.

TABLE 2

The Suppressive Effect of Purified Goat Anti-M104E on PFC in Murine Spleen Cells from SRBC-Immunized Animals

Experiment	Strain tested	Serum used for treatment of immune spleen cells	No. of direct PFC/10^6 cells[a]	No. of indirect PFC/10^6 cells[b]
1	BALB/c mouse	Purified NGS	228	905
		Purified Goat anti-M104E	0	20
2	C3H/He mouse	Purified NGS	62	
		Purified Goat anti-M104E	0	n.t.[c]
3	B10.D2 mouse	Purified NGS	84	
		Purified Goat anti-M104E	6	n.t.
4	Wister/Furth rat	Purified NGS	226	
		Purified Goat anti-M104E	242	n.t.

A 1:50 dilution of purified Goat anti-M104E or NGS was used for the treatment of spleen cells *in vitro*. Data are averages of triplicate determinations. Neither antiserum alone nor complement alone suppressed PFC.

[a] Spleen cells were assayed on the fifth day after ip immunization with 0.5 ml (in the case of mice) or 1.0 ml (in the case of rats) of 20% SRBC.

[b] Spleen cells were assayed on the fifth day after secondary ip immunization (0.2 ml of 20% SRBC) which was given 4 weeks after primary immunization.

[c] n.t.; not tested.

was killed by the antiserum, although the cytotoxic titer of the antiserum was similar to that against spleen cells of mice of other strains (titer 128 ~ 256).

The purified Goat anti-M104E was not cytotoxic for spleen cells of rats.

Cytotoxic Activity of Purified Goat anti-M104E against Splenic and Lymph Node B and T Cells

The evidence that a high proportion of spleen cells of nude mice is killed by the antiserum suggested that B cells are the cells which are susceptible to the cytotoxic activity of the antiserum. In order to test this possibility normal BALB/c mouse spleen cells or lymph node cells were separated into B and T cells by a nylon wool column as described in Materials and Methods and each cell population was tested for its susceptibility to the cytotoxic activity of purified Goat anti-M104E and, at the same time, also tested by anti-Thy-1.2 alloantiserum in order to check the purity of the cells in each population. As shown in Fig. 2a, B cells (nylon wool adherent cells) are efficiently killed by the purified Goat anti-M104E (maximum: 90%) but little affected by anti-Thy-1.2 antiserum (maximum: 15%). In contrast, T cells (nylon wool effluent cells) were refractory to purified Goat anti-M104E, a maximum of only 20% being killed, while about 85% of them were killed by anti-Thy-1.2 antiserum (Fig. 2b). The cytotoxic pattern of the purified Goat anti-M104E against T cells or B cells separated from lymph node cells or spleen cells of other strains of mice was the same as that against spleen cells of BALB/c mice.

Suppression of In Vitro Development of PFC by Purified Goat anti-M104E

The evidence that our purified Goat anti-M104E was also cytotoxic for nonmalignant plasma cells (PFC) in the spleen of an immune mouse was shown by the

TABLE 3

Removal of Suppressive Effect of Purified Goat Anti-M104E on PFC by Absorption with Thymus, Spleen and Myeloma Cells[a]

Serum used for treatment of spleen cells	No. of direct PFC/10^6 cells[b]
Purified NGS	142 ± 17
Purified Goat anti-M104E not further *in vitro* absorbed	2 ± 1
Purified Goat anti-M104E further absorbed with normal BALB/c thymus cells	46 ± 12
Purified Goat anti-M104E further absorbed with normal BALB/c spleen cells	196 ± 9
Purified Goat anti-M104E further absorbed with MOPC-104E cells	248 ± 29
Purified Goat anti-M104E further absorbed with PU-5(#16) cells	4 ± 2
Purified Goat anti-M140E further absorbed with X5563 cells	146 ± 45
Purified Goat anti-M104E further absorbed with 6C3HED cells	8 ± 3

[a] Spleen cells from primarily immunized BALB/c mice were treated with purified NGS or purified Goat anti-M104E (1:50) which had not been absorbed or was further *in vitro* absorbed with different cells. Absorption was done by incubating with an equal volume of packed, washed cells for 60 min at 4°C.

[b] Averages of triplicate determinations ± SE.

following experiment. Spleen cells obtained from the BALB/c mice previously immunized with SRBC were exposed to purified Goat anti-M104E in the presence or absence of rabbit complement and were then assayed for plaque formation. Treatment with the antiserum almost completely eliminated both direct and indirect PFC in a complement-dependent manner (Table 2). PFC of other strains of mice were also similarly suppressed by the treatment of antiserum plus complement but PFC of rat spleen were not suppressed at all.

Common Antigenicity of the Determinant on B Cells and Thymus Cells as Revealed by Absorption Tests

By cytotoxicity or PFC tests, the purified Goat anti-M104E revealed an antigenic determinant(s) which is expressed on mouse B cells, normal and malignant plasma cells, and a subpopulation of thymus cells (about 70%) but little, if any, on mature T cells. The common antigenicity of the determinant detected on both B cells and thymus cells by our antiserum was shown by the following absorption tests. The purified Goat anti-M104E diluted 1:32 or 1:64 was further absorbed *in vitro* with various mouse tissue or cells and tested for its residual activity by PFC or by cytotoxicity test against spleen cells, thymus cells and MOPC-104E cells, respectively. As shown in Table 3, the suppressive effect of purified Goat anti-M104E on PFC was abolished by absorption with myeloma cells (MOPC-104E and X5563) as well as normal BALB/c spleen cells. Normal BALB/c thymus cells weakly, but significantly, removed the activity also. Two T cell lymphomas [PU-5(#16) and 6C3HED] were not able to do so. In cytotoxicity tests (Tables 4a, b, c), the activity of the purified Goat anti-M104E could be efficiently removed by absorption with myeloma, spleen, lymph node, bone marrow and thymus cells irrespective of target cells tested (i.e., spleen cells, thymus cells and MOPC-104E myeloma cells). Cells which clearly failed to absorb cytotoxic antibodies and which therefore lack the antigen, were erythrocytes, three T cell lymphomas [PU-5(#16),

TABLE 4a

Absorption of Cytotoxic Activity of Purified Goat Anti-M104E Against Normal BALB/c Spleen Cells

Tissue used for absorption	% Spleen cells lysed by absorbed serum		Result of absorption
	Neat	1:1	
None	49	32	
BALB/c myelomas			
MOPC-104E, TEPC-183, McPC-774 ADJ-PC-22A, MOPC-315-Op, HOPC-8 TEPC-15, RPC-5, MOPC-21	<10	<10	++
C3H myeloma			
X5563	16	<10	+
BALB/c lymphoma			
P1798	29	11	+
PU-5(#16)	49	38	−
C3H lymphoma			
6C3HED	43	38	—
C57BL lymphoma			
EL-4	43	27	—
DBA/2 leukemia			
L1210	43	28	—
BALB/c normal tissues			
Erythrocytes	50	38	—
Liver	33	27	±
Kidney	17	15	+
Brain	32	18	±
Spleen cells	<10	<10	++
Lymph node cells	<10	<10	++
Bone marrow cells	<10	<10	++
Thymus cells	<10	<10	++
DBA/2 normal spleen cells	<10	<10	++

The purified Goat anti-M104E (1:32) was incubated with an equal volume of packed, washed cells or an equal volume of packed tissue sediment for 60 min at 4°C. The residual cytotoxicity of absorbed serum was tested against normal BALB/c spleen cells without further dilution (neat) or diluted (1:1).

6C3HED, and EL-4] and one Thy-1.2 negative leukemia (L1210). An interesting and unexpected finding was that one BALB/c T cell lymphoma, P1798, could weakly absorb cytotoxic antibodies although it was negative in the cytotoxicity test. The absorbing capacity of liver or brain tissues was questionable while that of kidney was very weakly positive.

In order to determine the relative amounts of antigenic determinant per cell for several different cell lines the purified Goat anti-M104E diluted 1 : 32 was absorbed with increasing numbers of cells. As shown in Table 5, the cytotoxic activity for spleen cells was most efficiently removed by homologous MOPC-104E cells. Only 5×10^6 MOPC-104E cells were needed to absorb the activity, while 5×10^7 and 5×10^8 of MOPC-315-Op and X5563 myeloma cells, respectively, were needed to do so. The cytotoxic activity was also removed by 5×10^8 cells from spleen, thymus or bone marrow of normal BALB/c mice. Splenic B cells were more potent than whole spleen cells and 10^8 cells were sufficient to absorb the antibodies. In contrast,

TABLE 4b

Absorption of Cytotoxic Activity of Purified Goat Anti-M104E Against Normal BALB/c Thymus Cells

Tissue used for absorption	% Thymus cells lysed by absorbed serum		Result of absorption
	Neat	1:1	
None	52	40	
BALB/c myelomas			
MOPC-104E, TEPC-183, McPC-774 ADJ-PC-22A, MOPC-315-Op, HOPC-8 TEPC-15, RPC-6	<10	<10	++
MOPC-21	16	<10	++
C3H myeloma			
X5563	30	30	±
BALB/c lymphoma			
P1798	44	23	±
PU-5(#16)	50	48	−
C3H lymphoma			
6C3HED	50	45	−
C57BL lymphoma			
EL-4	46	44	−
DBA/2 leukemia			
L1210	49	40	−
BALB/c normal tissues			
Erythrocytes	55	35	−
Liver	40	44	−
Kidney	39	30	±
Brain	46	33	−
Spleen cells	<10	<10	++
Lymph node cells	19	11	+
Bone marrow cells	32	18	+
Thymus cells	<10	<10	++
DBA/2 normal spleen cells	<10	<10	++

The purified Goat anti-M104E (1:32) was incubated with an equal volume of packed, washed cells or an equal volume of packed tissue sediment for 60 min at 4°C. The residual cytotoxicity of absorbed serum was tested against normal BALB/c thymus cells without further dilution (neat) or diluted (1:1).

10^8 splenic T cells failed to absorb and even 10^9 lymphoma cells of PU-5(#16) or 6C3HED were not able to absorb the activity. Almost the same patterns of quantitative absorption were obtained when the residual cytotoxic activity was tested against thymus cells.

Studies Utilizing Cell Fractionation on the FACS

In order to confirm that the antibodies in purified Goat anti-M104E serum were recognizing a common antigenic determinant on thymus and spleen cells, the antiserum was absorbed exhaustively *in vitro* with either BALB/cN spleen or thymus cells. The absorbed or unabsorbed antiserum samples were then assayed with spleen cells and thymus cells. The percent of the cells from each organ which reacted was determined on the FACS, following their staining with fluorescein labeled rabbit anti-goat Ig. The data (Table 6) show that the antibody reacting with spleen cells

TABLE 4c

Absorption by Various Tumors and Tissues of Cytotoxic Activity of Purified Goat Anti-M104E Against MOPC-104E Cells

Tissue used for absorption	% MOPC-104E cells lysed by absorbed serum		Result of absorption
	Neat	1:1	
None	>90	>90	
BALB/c myelomas			
MOPC-104E, TEPC-183, McPC-774 ADJ-PC-22A, MOPC-315-Op, HOPC-8 TEPC-15, RPC-5	<10	<10	+++
MOPC-21	47	<10	++
C3H myeloma			
X5563	85	<10	++
C57BL lymphoma			
EL-4	>90	>90	–
C3H lymphoma			
6C3HED	>90	>90	–
BALB/c lymphoma			
P1798	>90	39	+
DBA/2 leukemia			
L1210	>90	>90	–
BALB/c normal tissues			
Erythrocytes	>90	>90	–
Liver homogenate	>90	76	±
Kidney homogenate	>90	37	+
Brain homogenate	>90	>90	–
Spleen Cells	<10	<10	+++
Lymph node cells	71	<10	++
Bone marrow cells	19	<10	++
Thymus cells	<10	<10	+++
C3H/St/Ha spleen cells	<10	<10	+++

The purified Goat anti-M014E (1:64) was incubated with an equal volume of packed, washed cells or an equal volume of packed tissue sediment for 60 min at 4°C. Residual cytotoxicity of the thus absorbed serum was tested against MOPC-104E cells without further dilution (neat) or diluted (1:1).

could be completely removed by absorbing with thymus as well as spleen cells. The antibody reacting with thymus cells could be removed by spleen as well as thymus cells. These observations establish that the same antibodies are reacting with a determinant on spleen cells and on thymus cells.

In order to demonstrate that the spleen cells reacting with purified Goat anti-M104E antibodies were B cells and not T cells, spleen cells were labeled with rabbit antibodies to the brain associated T antigen (anti-BAT) and separated into BAT positive and BAT negative cells on the FACS. The two populations were then reacted with purified Goat anti-M104E serum, followed by fluorescein labeled rabbit anti-goat Ig. The percent of cells which were stained was determined on the FACS. The data (Table 7) show that essentially all the cells which were stained were BAT negative, i.e., were B cells and not T cells.

TABLE 5

Absorption by Various Tumors and Tissues of Cytotoxic Activity of Purified Goat Anti-M014E for Normal BALB/c Spleen Cells

Cells used for absorption	Cytotoxicity after absorption[a]							
	No. of absorbing cells used							
	10^5	10^6	5×10^6	10^7	5×10^7	10^8	5×10^8	10^9
MOPC-104E	+	+	–	–	–	–	–	–
MOPC-315-Op	+	+	+	+	–	–	–	–
X5563	+	+	+	+	+	+	–	–
Normal BALB/c whole spleen	+	+	+	+	+	±	–	–
splenic B cells	n.t.[b]	n.t.	n.t.	n.t.	n.t.	–	n.t.	n.t.
splenic T cells	n.t.	n.t.	n.t.	n.t.	n.t.	+	n.t.	n.t.
Normal BALB/c thymus	+	+	+	+	+	+	–	–
Normal BALB/c bone marrow	+	+	+	+	+	+	–	–
PU-5(#16)	+	+	+	+	+	+	+	+
6C3HED	+	+	+	+	+	+	+	+

The purified Goat anti-M104E (1:32) was incubated with a different number of cells for 60 min at 4°C. The residual cytotoxicity of absorbed serum was tested against normal BALB/c spleen cells.

[a] Cytotoxicity, +: positive (>80% of cytotoxicity of unabsorbed antiserum remained); –: negative (<20% of cytotoxicity of unabsorbed antiserum remained).

[b] n.t.; not tested.

Suppression of B Cell Function by Treatment with Purified Goat anti-M104E

The foregoing experiments showed that purified Goat anti-M104E can kill spleen or lymph node B cells but can hardly affect T cells. These results were functionally confirmed by the effect of the antiserum on the secondary response in cell transfer experiments. Spleen cells from BALB/c mice preimmunized with SRBC were treated with purified Goat anti-M104E plus complement *in vitro* and then transferred into irradiated syngeneic recipients along with SRBC. As controls, cells treated with purified NGS, anti-Thy-1.2 antiserum or normal AKR serum were used. Five days after cell transfer, recipients were killed and spleens were assayed

TABLE 6

In Vitro Absorption of Anti-Th-B Antibodies in Purified Goat Anti-M104E Serum with Thymus or Spleen Cells

Cells used for absorption	Percent of BALB/cN cells labeled	
	Spleen	Thymus
None	36	47
Thymus	3	<1
Spleen	<1	2

Purified Goat anti-M104E serum was exhaustively absorbed with either BALB/cN thymus or spleen cells. The absorbed sera as well as unabsorbed serum were used to label fresh BALB/cN spleen or thymus cells as described in the Materials and Methods section. The cells were analyzed on the FACS for percent of cells labeled.

TABLE 7

Reactivity of BAT+ and BAT− Spleen Cells with Goat Anti-Th-B Antibodies

Spleen cell fraction[a]	Percent cells reacted with[b]	
	Anti-BAT	Anti-Th-B
BAT+	94	4
BAT−	<1	82

[a] Spleen cells from 10 week old BALB/cN mice were reacted with rabbit antibodies against brain associated T antigen (BAT) and separated into BAT+ and BAT− fractions on the FACS. The separated fractions were then reacted with Goat anti-Th-B antibodies followed by fluoresceinated rabbit anti-goat Ig.

[b] Percentage of positive cells was determined by analyses on the FACS.

for the presence of direct and indirect PFC. As indicated in Table 8, development of both IgM (direct) and IgG (indirect) PFC in the Goat anti-M104E treated group were reduced to 44% and 17%, respectively, of control levels shown by cells treated with purified NGS. Anti-Thy-1.2 antiserum treatment also markedly suppressed the development of PFC, presumably due to the elimination of T cells. However, in the group which received a mixture of cells treated with purified Goat anti-M104E and cells treated with anti-Thy-1.2, the number of PFC observed were increased more than the sum of PFC in the two groups receiving each kind of treated cell alone, indicating that the Goat anti-M104E and anti-Thy-1.2 affected different populations of cells. Purified Goat anti-M104E removed B memory cells, and anti-Thy-1.2 removed T helper cells.

DISCUSSION

Since the discovery that two functionally different cells, T and B, are involved in the immune response (32, 33), much effort to find markers which distinguish between T cells and B cells has been made. As mentioned in the introduction, however, some markers which were thought to be exclusive B cell markers have now been found on at least some T cells also. The findings of these common properties

TABLE 8

Removal of B Memory Cells by Goat Anti-M104E

Immune spleen cells transferred[a]	PFC ± SE[b]/recipient spleen	
	Direct	Indirect
1×10^7 purified NGS treated cells	1,610 ± 300	41,790 ± 13,400
1×10^7 purified Goat anti-M104E treated cells	710 ± 460	7,200 ± 2,410
2×10^7 normal AKR serum treated cells	4,620 ± 1,660	67,200 ± 18,300
2×10^7 AKR anti-Thy-1.2 antiserum treated cells	740 ± 210	6,720 ± 1,860
1×10^7 purified Goat anti-M104E treated cells + 2×10^7 AKR anti-Thy-1.2 antiserum treated cells	1,760 ± 660	38,300 ± 11,100

[a] Mice were assayed at day 5 after cell transfer.

[b] Values represent average numbers of PFC ± standard errors from groups of five recipient mice.

of T and B cells may provide a clue to understanding the ontogeny and other characteristics of these cells.

We have previously reported (1) that an antiserum raised in rabbits against cells of the mouse myeloma MOPC-104E contains antibodies that can kill about half the population of normal mouse thymus cells as well as both normal and malignant plasma cells. This was due to a common antigenic determinant (Th-B) found on these cells. The antiserum has been also found to kill B cells but not T cells by cell transfer experiments (3) and to suppress the *in vivo* growth of different myelomas (34).

In the present studies, goat antiserum raised against MOPC-104E cells was used instead of rabbit antiserum because it could be shown to kill mouse B cells in spleen, lymph nodes and bone marrow by the direct cytotoxicity test. The rabbit antibody previously used was much less effective against these cells when examined by the direct cytotoxicity test. Thus the goat antiserum facilitated our analysis of the characteristics of antigenic determinant(s) recognized by the antibodies present. The purified goat antiserum was cytotoxic for B cells, normal plasma cells, plasma cell tumor cells as well as a major subpopulation of thymus cells. It was not cytotoxic for cells of most other non-plasma cell tumors tested and was least active against the T cell population of spleen or lymph nodes. Anti-Th-B antibodies were clearly shown by fluorescent labeling studies to bind to splenic B cells but not T cells. Irrespective of the target cells used, the cytotoxicity or the binding activity of the purified Goat anti-M104E antibodies was easily removed by absorption with tissues which contain B cells, plasma cells or thymus cells. Thus the common determinant Th-B is recognized by antibodies in Goat anti-M104E. Our previously reported (1, 2) rabbit anti-M104E antiserum contained some antibodies which bind to B cells only and not thymocytes. The Goat anti-M104E antiserum does not contain such antibodies since absorption of the antiserum with thymocytes removes all antibodies binding to B cells. Th-B is different from other lymphocyte surface antigens such as MBLA (10), PC-1 (12), MSLA (35), TL (36), Ly 1 (37) and Ly 2 (37) as previously discussed (1). A new alloantigen, Ly 4 (11, 38), which has recently been found to be predominantly represented on B cells is also different from Th-B since Th-B is found on B cells of all strains of mice and on a subpopulation of thymus cells whereas Ly 4 is an alloantigen present in only certain strains of mice and it is not found on thymus cells. I-region associated Ia antigens appear to be mainly found on B cells (39, 40), although recent detailed examination has detected their presence on T cells also (41, 42). However, Ia antigens are alloantigens and, therefore, are apparently different from Th-B.

Almost 100% of mature B cells may be regarded as possessing Th-B since the purified Goat anti-M104E could kill approximately 90% of spleen cells from nude mice. The remaining 10% of cells may be a type other than lymphocytes. In addition, 90% of the cells in the B cell fraction of spleen or lymph node from normal mice as separated by nylon wool columns contain Th-B. Therefore, the evidence that only about 20% of bone marrow cells are killed by purified Goat anti-M104E may suggest that only a small percentage of bone marrow cells are mature B cells and a majority of them are precursors which express little Th-B on their surfaces. Consistent with this interpretation is the finding that bone marrow contains many null cells on which neither a B cell nor a T cell marker is detectable (43, 44) and that

such cells can mature into B cells in the peripheral lymphoid tissues (44). IgM-producing myeloma cells were more efficiently killed by purified Goat anti-M104E when compared with other lines of myeloma cells, suggesting that IgM-producing cells possess a greater amount of Th-B. From these results, it may be suggested that B cells possess different amounts of Th-B depending on their maturity.

Mature T cells separated from spleen or lymph node cells were least susceptible to the purified Goat anti-M104E whereas about 70% of thymus cells were killed. These findings taken together with the observations that even T cells probably originate in the bone marrow in adults (21–23) suggest to us that Th-B starts to develop on the stem cells which are common to B and T pathways and that Th-B is fully expressed on immature cells in the thymus but is lost from these cells as they mature. The acquisition of Th-B may not be influenced by the thymus because even nude mice develop Th-B; instead the thymus may influence the disappearance of Th-B from T cells.

One of the T cell lymphomas, BUGSY 40 was judged to possess Th-B from its weak but definite susceptibility to the cytotoxicity of purified Goat anti-M104E. Another T cell lymphoma P1798 was also Th-B positive in the absorption test in spite of being negative in the cytotoxicity test. The discrepancy between the presence of Th-B on P1798 and BUGSY 40 cells and its absence on other T cell lymphoma cells may be explained by differences in the differentiation stage of cells in the thymus from which T cell lymphomas originate. Another possibility is that P1798 and BUGSY 40 are representative of the cells which possess a mixture of B and T cell characteristics. In fact, such cells have been identified in man (45) and in the mouse (46). A less likely explanation for the observation of Th-B in lymphoma P1798 may be that the tumor contains a small fraction of B cells from normal tissue and the latter furnished the antigen responsible for the absorption.

Plasma cells, both normal and malignant, are also known to possess the alloantigen PC-1 (12) or a xenoantigen (13) both of which are commonly expressed on liver, kidney and brain but not on T cells, including thymus cells. These antigens seem to be more selectively expressed on plasma cells than on B cells (47 and our unpublished results). Thus the tissue distribution of these antigens is apparently different from Th-B. It will be of interest to find the relationship between Th-B and the antigen(s) common to plasma cells, liver, kidney and brain cells since kidney cells were found to be weakly Th-B positive in the present study. The presence of Th-B in liver and brain was questionable. In addition, in our experience one goat (#68) which was also immunized with MOPC-104E cells produced an antiserum which has specificities against the plasma cell-liver-kidney-brain common antigen (unpublished observation). The procedure of immunization and purification of the antiserum were similar to those used for the antiserum from the goat (#72) utilized in the present study which produced anti-Th-B antibodies, except that an older generation of MOPC-104E cells were used when goat #68 was immunized. The same specificity of antiserum as that produced by goat #68 was also obtained in a goat antiserum raised against cells of MOPC-21, which had been producing IgG, but changed into nonparaprotein producing myeloma, and a goat antiserum against cells of ADJ-PC-22A, an IgA-producing myeloma (unpublished).

The present goat anti-Th-B antiserum may offer the following advantages over other means of eliminating B cells from mixed populations of lymphoid cells; (a)

a large amount of antiserum is available, (b) it may be purified by a rapid, easy and reliable procedure of *in vivo* absorption, (c) it is applicable to all strains of mice (not strain-specific), and (d) elimination of B cells can be accomplished rapidly in the presence of complement.

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