SURFACE ALLOANTIGENS OF PLASMA CELLS*‡

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(Received for publication 28 January 1970)

Recently there have been gratifying advances in the identification and quantitation of surface antigens on mouse thymocytes, lymphocytes, and leukemia cells (1). These cells have been studied more than others from this point of view because they can readily be obtained viable in free suspension without the need for enzymatic or mechanical dispersion. Briefly, this is what is known:

There are so far three sets of cell surface antigens found only on thymocytes and lymphocytes; they are MSLA (mouse specific lymphocyte antigen)[1] (2), a species antigen recognized by absorbed rabbit anti-thymocyte and anti-lymphocyte sera, and two genetically unlinked sets of alloantigens, Ly-A and Ly-B (3).

Thymocytes have more of these three antigens than do lymphocytes, and also of the alloantigen θ (4) which is expressed also on brain. Information on the existence and tissue representation of other alloantigens serologically detectable on lymphoid cells is accumulating.

In those strains of mice that express TL antigens (TL^+ strains) these are restricted to thymocytes; this absolutely distinguishes the thymocyte from all other lymphocytes in TL^+ strains (see reference 1).

H-2 alloantigens (5), which occur on most cell types, are present in much higher amounts on lymphocytes than on thymocytes, in contrast to all the antigens mentioned above (1).

It has long been suspected, on various grounds, that lymphocytes, although they are morphologically uniform, are diverse in origin and function. What can we expect the study of the surface antigens to tell us about this probable heterogeneity of lymphocytes? To begin with, we know that all or a portion of lymphocytes from different anatomical sites share the antigens referred to above. We also recognize that thymocyte populations can be distinguished from lymphocyte populations by differences in the amount of any one of these antigens, as well as by the absolute restriction of TL antigens to thymocytes. If some lymphocytes are derived from thymocytes, which is still a subject of controversy, considerable remodeling of the cell surface must take

* The authors' work is supported in part by National Cancer Institute Grant CA 08748 and a grant from the John A. Hartford Foundation, Inc.

‡ There may be some debate about the extent to which the terms "plasma cell," "immunoglobulin-forming cell," and "hemolytic plaque-forming cell" are interchangeable; throughout the text we have used whichever seemed most appropriate in its context.

[1] Abbreviations used in this paper: MSLA, mouse specific lymphocyte antigen; EBSS, Earle's balanced salt solution; PFC, plaque-forming cells; *Pca-plasma cell antigen.*

place in the process, involving loss of TL, decrease of θ, Ly-A, Ly-B, and MSLA, and a fourfold increase of H-2. The antecedent differentiation of bone marrow stem cells to thymocytes involves perhaps an even more extensive revision of surface structure, for probably none of these antigens except H-2 is expressed on the stem cell (1).

Turning to the question of cell surface antigens expressed on malignant cells, leukemias carry four known classes of cell surface antigens: (*a*) the normal species antigens and alloantigens already mentioned, (*b*) TL antigens coded by derepressed *Tla* genes of the cellular genome, (*c*) antigens coded by oncogenic viruses, including the various leukemia viruses and a variant of the mammary tumor virus, and (*d*) other antigens, such as "E," whose specification is not sufficiently clear to permit relegation to one of the preceding three categories (6, 7).

We have proposed elsewhere that TL, θ, Ly-A, Ly-B, and MSLA are "differentiation" antigens, owing their expression to selective gene action peculiar to lymphoid differentiation, and that they may serve as markers identifying surface elements necessary to the functional or behavioral properties of the lymphoid cell (1). An important extension of this hypothesis is that cell recognition in general is implemented by similar surface structural codes, via selective gene action.

To substantiate this in serological terms at the present time is difficult, mainly because other tissues cannot in general be analyzed immunogenetically in quite the same way as lymphoid cells. However, several types of malignant cell populations are amenable to many of the relevant procedures, and of these tumors the transplanted myeloma (plasma cell tumor) is of special interest because of its suspected but undefined relation to the thymocyte-lymphocyte axis, about which a great deal is already known.

Despite the handicap of being denied a homogeneous population of normal cells to work with (because it is impracticable to prepare homogeneous suspensions of normal viable plasma cells) we have been able to establish a number of facts that bear on surface structure in relation to the differentiation of plasma cells, normal and malignant, and these are reported here. We set out to investigate two questions: (*a*) Are the cell surface antigens characteristic of thymocytes and lymphocytes expressed on plasma cells? (*b*) Do plasma cells have their own special cell surface alloantigens?

Materials and Methods

Mice.—These were obtained either from our colonies or from the Jackson Laboratory. (Abbreviations: BALB = BALB/c; C57BL = C57BL/6 except in reference to the transplanted C57BL ascites leukemia, EL4, whose subline of origin is not known to us).

Transplanted Tumors.—Myelomas: See reference 8. Table I identifies the seven transplanted plasma cell tumors included in this study. *Leukemias and sarcomas*: See previous publications from this laboratory (9–11).

Antisera.—(*a*) See Table II for antisera used to identify known antigens of thymocytes and lymphocytes. (*b*) Antiserum to the BALB (H-2^d) myeloma MOPC-70A, identifying the new alloantigen PC.1 (see Results), was prepared in female DBA/2 mice (also H-2^d) by three or more inoculations of MOPC-70A, beginning with 1–5 $\times$ 10^5 viable cells subcutaneously, followed 3 wk later by 1–5 $\times$ 10^6 cells subcutaneously and then 5 $\times$ 10^6 intraperitoneally

at intervals of 3 wk; the recipients were bled from the tail under ether anesthesia 7–10 days after the last inoculation and their serum pooled. Erythrocytes and other host cells were largely eliminated from MOPC-70A used for immunization by several cycles of centrifugation at 400 rpm for 10 min in an International Centrifuge (Model PR-2, International Equipment Co., Needham Heights, Mass.).

TABLE I

The Myelomas

Description	Mouse strain of origin	Immunoglobulin product	Carried as ascites or subcutaneous transplant	Source
MOPC-70A	BALB	$\gamma_1\kappa$	Ascites	Dr. M. N. Teller, Sloan-Kettering Institute
MOPC-104E	"	$\mu\lambda + \lambda$	Ascites	
MPC-67	"	$\alpha\kappa$	Ascites	
MPC-11	"	$\gamma_{2b}\kappa$	Subcutaneous	Dr. J. W. Uhr, New York University
Adj-PC5	"	$\gamma_{2a}\kappa$	Subcutaneous	Dr. T. A. Conners, Chester-Beatty Research Institute
X5563	C3H/He	$\gamma_{2a}\kappa$	Subcutaneous	Dr. M. Potter, National Institutes of Health
MOPC-387	(BALB × DBA/2)F_1	$\alpha\kappa$	Subcutaneous	

TABLE II

Antisera Recognizing Surface Antigens on Cells of the Thymocyte-Lymphocyte Axis

Serum	Cell surface antigens recognized
C57BL anti BALB ascites sarcoma Meth A	$H\text{-}2^d$
A/TL⁻ (congenic with A) anti A strain spontaneous leukemia ASL1	TL.1, 2, 3
AKR anti C3Hf/Bi thymocytes	θ-C3H
DBA/2 anti C57BL ascites leukemia EL4 (test cells: BALB thymocytes)	Ly-A.2 (on BALB thymocytes)
C57BL/Ly-B.1 (congenic with C57BL) anti C57BL radiation-induced leukemia ERLD	Ly-B.2
Rabbit anti A strain thymocytes, (absorbed in vivo in A strain mice)	MSLA

Cell Suspensions.—(*a*) Cells from thymus, lymph node, and spleen were obtained by mincing these tissues with curved scissors in a few drops of Medium 199, the released cells having been washed twice before use. (*b*) Blood lymphocytes: One part of heparinized blood and 3 parts of Plasmagel (Laboratoire Roger Bellon, Seine, France) were incubated separately for 30 min at 37°C, then mixed and left for 45–60 min at 37°C. After sedimentation of erythrocytes, the cells in the supernatant fluid were washed twice by centrifugation at 1000 rpm

for 10 min at room temperature and resuspended in Medium 199; about 90% of the nucleated cells in this final preparation were lymphocytes. (*c*) Peritoneal lymphocytes: Cells were collected from the peritoneal cavity by lavage with Medium 199 supplemented with 10% fetal calf serum and heparin; after two washings in the same medium, the cells were resuspended in a concentration of 3×10^7/ml in Medium 199 plus 20% fetal calf serum and then incubated in a flat-bottomed tissue culture flask for 10 min at 37°C. Under these conditions macrophages adhere to the glass surface while the lymphocytes remain unattached. After three or more transfers of the unattached cells to new culture vessels the final suspension comprised >90% lymphocytes; these were washed once in Medium 199 before use.

Liver, Kidney and Brain Homogenates.—Tissues from exsanguinated mice were minced with curved scissors and gently homogenized in a Teflon tissue homogenizer (TRI-R Instruments, New York) in Earle's balanced salt solution (EBSS). After the large tissue fragments settled, the cells in the supernatant fluid were collected by centrifugation and washed three or more times to remove cell fragments that were not brought down by centrifugation for 10 min at 2000 rpm. The washed cells were then resuspended in EBSS, transferred to small glass tubes (6 × 50 mm), and centrifuged at 2000 rpm for 15 min. These pellets of packed cells were used for absorption of antiserum.

Cytotoxic Test.—Based on Gorer and O'Gorman (12) with modifications (13). 0.05 ml volumes of (*a*) cells (5×10^6/ml), (*b*) antiserum (serially diluted), and (*c*) guinea pig serum (complement source, diluted 1:3 or 1:4) were incubated in (10 × 75 mm) tubes for 45 min at 37°C. The proportion of dead cells was determined by adding 0.1 ml of 0.16% trypan blue to each tube. The dye stains dead cells but is excluded by living cells.

Absorption Test for Determining the Presence or Absence of an Antigen.—See reference 11. The cytotoxic end point of the relevant antiserum (50% cells dead) is first determined by titration (twofold dilutions) against the most sensitive available test cell (e.g. lymphocytes in the case of H-2 and thymocytes in the case of θ). A dilution one to two tubes below this is selected for the absorption tests. Equal volumes of washed packed cells or homogenized tissues are incubated for 30 min at 4°C with shaking. The absorbed antiserum is recovered after centrifugation in the cold and tested in serial dilution for residual cytotoxic activity against cells of the relevant type.

Estimation of the Amount of a Cell Surface Antigen by Quantitative Absorption.—See reference 3. Aliquots (0.06 ml) of appropriately diluted antiserum (see preceding paragraph) are absorbed with a series of counted numbers of the washed viable cells to be tested, spanning the range from undetectable to complete absorption. The absorbed antiserum aliquots are then titrated with the appropriate test cell for residual cytotoxicity. The residual cytotoxic activity, expressed as per cent of cells stained, is plotted against numbers of cells used for absorption to give an absorption curve. The absorption index is the number of cells (n) indicated by the intersection of this curve with the level of 50% cells dead. Thymocytes were included as the standard reference cells in every test, and the antigen content of other cells was expressed as per cent antigen content of thymocytes.

$$\text{Antigen content (relative to thymocytes)} = \frac{n \text{ (thymocytes)}}{n \text{ (cells being tested)}} \times 100$$

Assays for γM or γG Plaque-Forming Cells (PFC).—Adult mice were injected intravenously with 0.2 ml of 20% washed sheep erythrocytes in EBSS; this was repeated 3 wk later in the case of the γG PFC assay. For γM-forming PFC assay (14) spleen cells were obtained on the 4th day after primary immunization; γG-forming PFC were assayed according to Dresser and Wortis (15) with rabbit anti-mouse γG diluted 1:150 and spleen cells obtained 5 days after secondary immunization.

Detection of Cell Surface Antigens on PFC.—The spleen cells were washed twice and sus-

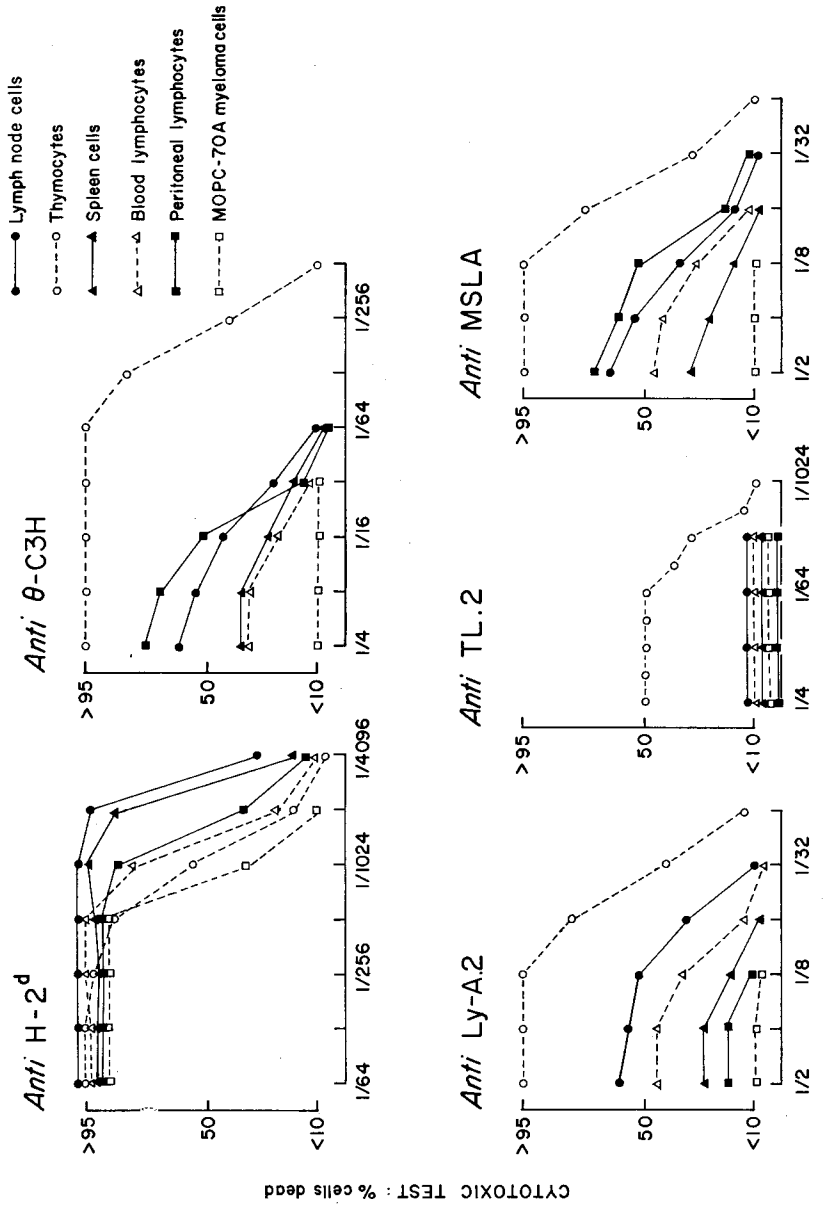

FIG. 1. Cytotoxic activity of H-2, θ, Ly-A, TL, and MSLA antisera on thymocytes, on lymphocytes from various sources, and on MOPC-70A myeloma cells, (source of complement: guinea pig serum). The cytotoxic activity of anti Ly-B.2 (not shown) was similar to that of Ly-A.2. Conclusion: Only H-2 is demonstrable on the myeloma cells; antigens of the other five systems, which are differentiation antigens of thymocytes and lymphocytes, are apparently absent.

pended in Medium 199 containing 2% fetal calf serum. Tubes containing equal volumes (0.1 ml) of: (*a*) cells (approximately 1–2 × 10^6); (*b*) antiserum diluted 1:10, or normal mouse serum; and (*c*) rabbit serum[2] diluted 1:15 (complement source) or guinea pig serum 1:3 (alternate complement source, see Results), were incubated together at 37°C for 45 min in serological tubes. The cells in each tube were then washed once, resuspended in a volume approximately 0.1 ml, and added to a single agar plate for assay of PFC (see above).

RESULTS

Section I

Tests for H-2, θ, TL, Ly-A, Ly-B, and MSLA Antigens on BALB Myeloma Cells.—The three ascites myelomas MOPC-70A, MOPC-104E, and MPC-67

TABLE III

Summary of Quantitative Estimations of θ, Ly-A, and MSLA Antigens on BALB Thymocytes, Lymphocytes, and Myeloma Cells

Cells	θ-C3H	Ly-A.2 Amount of Antigens*	MSLA
	%	%	%
Normal BALB			
Thymocytes	100	100	100
Lymph node cells	25	66	46
Spleen cells	16	55	25
Blood lymphocytes	19	68	32
Peritoneal lymphocytes	21	52	52
BALB myeloma			
MOPC-70A, MOPC-104E, MPC-67	<5	<5	<5

* Absorption capacity (see Fig. 2) relative to thymocytes (thymocytes content = 100%).

were tested for sensitivity to antisera of all these six systems and were found to be resistant to all of them except anti H-2. BALB thymocytes, as expected, were sensitive to antisera of all six systems. Lymphocytes from spleen, blood, peritoneal cavity, and thoracic duct were sensitive to all antisera except anti TL. Some representative results are illustrated in Fig. 1.

Absorption of antibody from antiserum diluted to the region of the endpoint (see Methods) is a sensitive test for a cellular antigen present in amounts too low to render the cell sensitive to immune cytolysis by the respective antibody and complement. Also according to this criterion all the antigens named above, with the exception of H-2, were absent from the three myeloma lines. In some instances, quantitative absorption tests were performed on thymocytes,

[2] Footnote: From a pool of serum from rabbits selected for low toxicity to mouse cells.

lymphocytes from various sources, and the three myeloma cells (Fig. 2 and Table III). These results confirm the absence of all except H-2 from the three myeloma lines, the presence of all except TL on lymphocytes from all available sources, and the presence of all six antigens, without exception, on thymocytes.

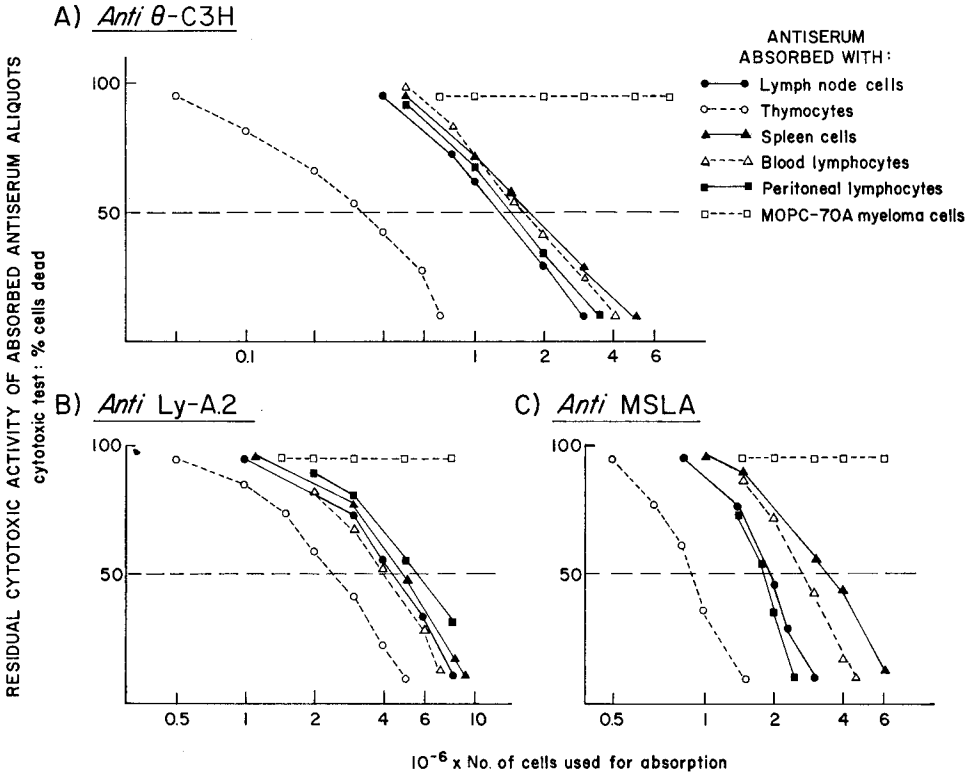

FIG. 2. Quantitative estimation of θ, Ly-A, and MSLA antigens on MOPC-70A myeloma cells, on thymocytes, and on lymphocytes from various sources. Conclusion: These antigens are not demonstrable on MOPC-70A myeloma cells (illustrated here) or on two other myelomas. MOPC-104E and MPC-67, (not shown in this Figure). Other absorption tests have given similar negative results for TL and Ly-B antigens.

Section II

The PC.1 (Plasma Cell 1) Alloantigen Defined by the Serum DBA/2 anti BALB Myeloma MOPC-70A.—The formation of non-H-2 antibodies is sometimes facilitated by the choice of an H-2-compatible donor–recipient pair for immunization. This is why the antiserum DBA/2 anti BALB myeloma MOPC-70A was included among several immunizations performed during the search

for antigens peculiar to myeloma cells and normal plasma cells. DBA/2 and BALB are both H-2^d and although they differ from Ly-A and Ly-B it was not anticipated that antibodies to these antigens would be formed, because neither is carried on the myeloma cells used for immunization (see Section I). In fact anti Ly-A.2 and anti Ly-B.2 were not elicited, as can be seen from Fig. 3, since no cytotoxic activity is present against BALB thymocytes and lymphocytes, but the antiserum was strongly cytotoxic for all three myelomas (Fig. 3). The specificity of this newly recognized reaction was distinguished from the leukemia-associated antigens G, FMR, ML, E, and TL by lack of cytotoxicity for the standard leukemias bearing these antigens which we maintain for typing purposes.

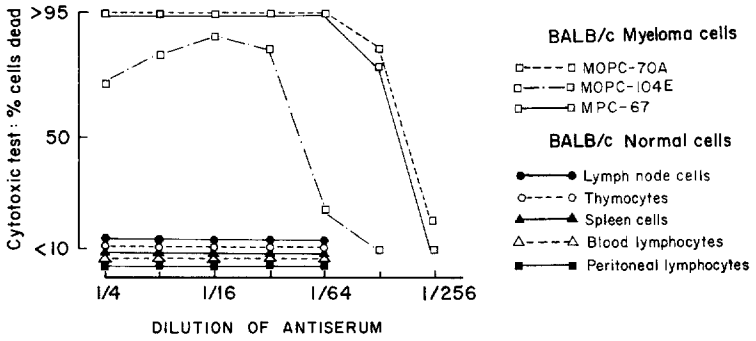

FIG. 3. Cytotoxic activity of DBA/2 anti MOPC-70A serum. This antiserum is not cytotoxic for thymocytes or lymphocytes; the specificity of the reaction against the myeloma lines is anti PC.1 alloantigen.

Absorption tests (Table IV) showed the antigen to be present on all five myelomas tested and also in some normal tissues of BALB mice: liver, kidney, brain, spleen, and, in lesser amount, lymph node cells. Absorption of anti PC.1 by liver, kidney, and brain cannot be ascribed to their content of lymphoid cells because the absorption capacity of these tissues was not reduced by massive pretreatment of the donors with corticosteroid (Dexamethasone 4 mg/kg per day for 5 days). Specificity is shown by lack of absorption by the same tissues of DBA/2 origin. BALB tissues which failed to absorb cytotoxic antibody, and which therefore lack the antigen, are thymocytes, peritoneal lymphocytes, blood lymphocytes, bone marrow cells, erythrocytes, two leukemias, and an ascites sarcoma.

Cytotoxicity cannot be attributed to reaction with the $\gamma 1$ immunoglobulin which the immunizing myeloma MOPC-70A produces and may bear on its surface because (*a*) the strain distribution of the antigen (Table V) does not correspond to any known $\gamma 1$ allotype, (*b*) the serum of normal BALB mice or BALB mice bearing MOPC-70A did not neutralize cytotoxic antibody or

precipitate with the antiserum, and (*c*) the antiserum was cytotoxic for BALB myelomas producing γA or γM as well as for the γ1-producing MOPC-70A.

The typing of mice of various strains for the presence or absence of this new antigen was done by absorption with liver, a convenient plentiful source, and testing the absorbed serum against MOPC-70A cells, as illustrated in Table IV.

TABLE IV

Tissue Representation of PC.1 Antigen Determined by Absorption of Cytotoxic Antibody

DBA/2 anti MOPC-70A serum absorbed with:	%MPOC-70A cells lysed by absorbed serum		Result of absorption
	1:1*	1:2	
BALB myelomas			
MOPC-70A, MOPC-104E, MPC-67, MPC-11, or Adj-PC5	<10	<10	+
BALB leukemias			
BALB♂RL2, or BALB♂SL2	>95	>80	−
BALB ascites sarcoma			
Meth A	>95	91	−
BALB normal tissues			
Liver or kidney homogenate	<10	<10	+
Brain homogenate	23	<10	+
Spleen cells	12	<10	+
Lymph node cells	73	37	±
Peritoneal or blood lymphocyte	—	>95‡	−
Cells from thymus or bone marrow; erythrocytes	>95	>95	−
DBA/2 normal tissues (negative control)			
Liver homogenate	>95	89	−
Kidney homogenate	>95	78	−
Brain homogenate; or cells from thymus, spleen or lymph nodes; or erythrocytes	>95	>95	−

Method: 0.2 ml DBA/2 anti MOPC-70A 1:20 incubated with 0.2 ml of packed, washed cells or tissue homogenate for 30 min at 4°C.

* Residual cytotoxicity of absorbed serum tested against MOPC-70A cells without further dilution (1:1) or diluted 1:2.

‡ For peritoneal lymphocytes and blood lymphocytes (obtainable only in relatively small numbers) 0.06 ml of antiserum diluted 1:40 was absorbed with 10^7 cells.

The strain distribution does not correspond with that of any known cell surface alloantigen or serum protein allotype. C3Hf/Bi differs from C3H/An and C3H/He, providing a third serologically demonstrable antigenic difference between these sublines, the other two being H-6 (17) and the antigens specified by the mammary tumor virus.

Typing of segregating progeny of backcross and F_2 generation from a cross between PC.1$^+$ and PC.1$^-$ strains gave clear-cut positive or negative results for individual mice, and the ratios are characteristic of a single mendelian dominant gene (Table VI). We have designated the locus *Pca* (*plasma cell antigen*).

Section III

Tests for PC.1, H-2, θ, Ly-A, Ly-B, and MSLA Antigens on Normal Immunoglobulin-Forming Cells.—Leukemia cells commonly carry the normal surface antigens of the normal lymphoid cells from which they are descended. Thus it is inferred that any normal antigens expressed on myeloma cells will also be expressed on normal plasma cells. Accordingly it might be anticipated

TABLE V

*Strain Distribution of PC.1 Antigen**

PC.1+	PC.1−
C3H/An	C3Hf/Bi
C3H/HeJ	C57BL/6
A	C57BL/H-2^k (congenic)
A/TL− (congenic)	C57BL/TL+ (congenic)
A.SW	C57BL/Ly-A.1 (congenic)
AKR	C57BL/Ly-B.1 (congenic)
AKR/H-2^b (congenic)	B10.D2 (congenic)
BALB/c	H-2I
BALB/c-2 (congenic)‡	H-2G
NZB/Bl	C58
MA/J	CBA-T6 (Lyon)
RF/J	DBA/2
101	129
SJL/J	I
PL	
CE	

* Typing by absorption of DBA/2 anti MOPC-70A serum (anti PC.1) with washed liver homogenate; absorbed antiserum tested for residual cytotoxicity on MOPC-70A cells. Liver was chosen as the tissue for absorption because it provides a conveniently large amount of material.

‡ Indicating independence of the *Pca* and *Ig* loci. BALB/c-2 mice (kindly provided by Dr. Michael Potter) differ from the congenic partner BALB/c by possessing the C57BL/6 Ig heavy chain allotype. (See reference 16.)

that normal BALB immunoglobulin-forming cells will express H-2 and PC.1 antigen but not the characteristic lymphocyte differentiation antigens because these latter are known to be absent from myeloma cells (Section II).

This was verified by testing hemolytic plaque-forming cells (PFC) of BALB spleen for their sensitivity to the relevant cytotoxic antisera. For this purpose spleen cells from BALB mice immunized against sheep erythrocytes were incubated with each antiserum in the presence of complement as in the conventional cytotoxic test. After washing, the cells were tested for their capacity to produce hemolytic plaques in agar plates containing sheep erythrocytes (see Methods). H-2 antiserum was lethal for both γM-producing and γG-producing PFC in the presence of guinea pig serum, but under the same conditions PC.1

antigen was demonstrable only on γG-producing PFC (Table VII). It seemed unlikely that this distinction was genuine, for PC.1 antigen is carried by the γM-producing myeloma MOPC-104E. Accordingly the experiments were repeated with guinea pig serum replaced by rabbit serum, which is known to be a superior source of complement in mouse cytotoxic assays (reviewed by Haughton and McGehee, 18), and the distinction was no longer apparent (Table VIII). A comparison of Tables VII and VIII provides a remarkable further illustration of superior complementation by rabbit serum in comparison with guinea pig serum.

TABLE VI

Independent Segregation of H-2 and Pca Genes in Backcross and F_2 Populations from the Cross C57BL ($H\text{-}2^b$:PC.1$^-$) × BALB ($H\text{-}2^d$:PC.1$^+$)

Population		PC.1$^+$	PC.1$^-$	Total
Backcross to C57BL				
$H\text{-}2^{d+}$		11	14	25
$H\text{-}2^{d-}$		13	14	27
	Total	24	28	52*
F_2 generation				
$H\text{-}2^{dd}$		5	4	9
$H\text{-}2^{bd}$		17	3	20
$H\text{-}2^{bb}$		9	2	11
	Total	31	9	40‡

* 28 ♀♀ and 24 ♂♂.
‡ 21 ♀♀ and 19 ♂♂.

To make sure that the suppression of PFC produced by anti PC.1 antiserum was not due to an unrecognized cytotoxin in the PC.1 antiserum DBA/2 anti MOPC-70A the following test of specificity was carried out:

Progeny of a PC.1$^+$ to PC.1$^-$ backcross were typed PC.1$^+$ and PC.1$^-$ by absorption with liver (see text and Table V). The PC.1$^+$ livers and the PC.1$^-$ livers were then separately pooled and used to absorb two aliquots of anti PC.1 antiserum. The two absorbed antisera were then tested for anti PFC activity as in Table VIII. The antiserum absorbed with the PC.1$^-$ pool retained activity against both γG and γM PFC. The antiserum absorbed with the PC.1$^+$ pool lost both activities. This shows that the anti PFC (both γM and γG) activity of DBA/2 anti MOPC-70A is due to anti PC.1.

Anti PFC activity by anti PC.1 serum was weaker than by anti H-2 serum but there is no reason to attach more significance to this than a difference in the cytotoxic potency of the two antisera under the conditions obtaining.

Antisera of the four systems θ, Ly-A, Ly-B, and MSLA (anti TL was not tested) had no significant anti PFC activity.

TABLE VII

Tests for the Presence of H-2 and PC.1 Antigens on Hemolytic Plaque-Forming Cells (PFC). Inhibition of Plaque Formation by Preincubation of PFC with H-2 or PC.1 Antiserum in the Presence of Guinea Pig Complement

BALB spleen cells (PFC) preincubated with*	γM PFC						γG PFC					
	Exp. 1			Exp. 2			Exp. 1			Exp. 2		
	No. of plaques	Total plaques	Standard	No. of plaques	Total plaques	Standard	No. of plaques	Total plaques	Standard	No. of plaques	Total plaques	Standard
		Σ§	%‡		Σ	%		Σ	%		Σ	%
BALB ($H\text{-}2^d$) anti $H\text{-}2^a$ (standard negative control)	52, 44, 29	125	100	49, 53, 45	147	100	52, 56, 60	168	100	51, 41, 42	134	100
DBA/2 normal mouse serum	41, 32, 46	119	95	53, 47, 44	144	98	59, 64, 47	170	101	32, 46, 48	126	94
Anti PC.1	34, 47, 48	128	102	48, 55, 55	158	108	17, 18, 11	46	27	6, 5, 13	24	20
Anti $H\text{-}2^d$	0, 0, 2	2	2	3, 0, 1	4	3	5, 1, 2	8	5	2, 3, 1	6	4

Conclusion: Both $H\text{-}2^d$ and PC.1 antigens are present on γG-producing PFC of BALB spleen. On γM-producing PFC $H\text{-}2^d$ was demonstrable but not PC.1; but see Table VIII showing that PC.1 is demonstrable on these also when rabbit serum rather than guinea pig serum is the complement source.

* Dilution of all antisera 1:10; conditions of preincubation of spleen cells, as for cytotoxic tests.

‡ ΣPFC/(ΣPFC with standard negative control antiserum $H\text{-}2^d$ anti $H\text{-}2^a$) × 100.

§ Total plaques in three similar plates (sum of the three preceding figures).

TABLE VIII

Tests for the Presence of H-2, θ, Ly-A, Ly-B, MSLA, and PC.1 Antigens on Hemolytic Plaque-Forming Cells. Inhibition of Plaque Formation by Preincubation of PFC with H-2 or PC.1 Antiserum in the Presence of Rabbit Complement

BALB spleen cells (PFC) preincubated with*	γM PFC						γG PFC					
	Exp. 1			Exp. 2			Exp. 1			Exp. 2		
	No. of plaques	Total plaques	Standard	No. of plaques	Total plaques	Standard	No. of plaques	Total plaques	Standard	No. of plaques	Total plaques	Standard
		Σ§	%‡		Σ	%		Σ	%		Σ	%
BALB ($H\text{-}2^d$) anti $H\text{-}2^a$ (standard negative control)	52, 41, 36	129	100	83, 69, 73	225	100	37, 38, 56	131	100	65, 82, 62	209	100
DBA/2 normal mouse serum	44, 47, 39	130	101	82, 70, 66	218	99	65, 42, 48	155	108	71, 75, 81	227	108
Anti θ-C3H	42, 37, 53	132	102	67, 68, 61	196	87	55, 47, 37	139	106	89, 63, 84	236	113
Anti Ly-A.2	44, 46, 47	137	106	59, 77, 76	212	94	40, 39, 46	125	95	73, 53, 78	204	98
Anti Ly-B.2	45, 46, 59	150	112	69, 58, 71	198	88	46, 42, 36	124	95	64, 62, 73	199	95
Anti MSLA	52, 46, 43	141	109	73, 72, 66	211	94	51, 54, 48	153	117	81, 92, 73	246	117
Anti PC.1‖	17, 27, 26	70	54	16, 27, 22	65	29	14, 8, 9	31	24	13, 12, 19	44	21
Anti $H\text{-}2^d$	3, 6, 5	14	11	4, 3, 3	10	4	6, 1, 2	9	7	6, 2, 4	12	6

Conclusion: Both $H\text{-}2^d$ and PC.1 antigens are present on both γM-producing and γG-producing PFC of BALB spleen but antigens θ, Ly-A, Ly-B, and MSLA are not demonstrable on either type of cell.

* ‡ § See footnotes to Table VII.

‖ For specificity controls see text.

DISCUSSION

The cell which gives rise to the plasma cell is not absolutely identified. Four contenders that have been considered are lymphatic reticular cells (19–21), lymphocytes (22–24), endothelial cells in the postcapillary venules of lymphatic tissue (25), and perivascular mesenchymal cells (26). The ancestral cell, not necessarily the immediate parent cell, is assumed to be a mobile cell of the bone marrow, for immunoglobulins of allogeneic radiation chimeras are of the donor type (27).

A further indication of the cellular origin of plasma cells is the presence or absence of the cell surface antigens characteristic of lymphocytes. These include the three sets of alloantigens (θ, Ly-A, and Ly-B) and the species antigen MSLA, which occur on all mouse lymphocyte populations (not necessarily on all individual lymphocytes) whatever their origin, whether from thymus, lymph nodes, spleen, peritoneal cavity, peripheral blood, or thoracic duct.[3] Leukemia cells often continue to express these differentiation antigens even after years of serial transplantation; their presence is probably the most certain indication that a given malignant cell line originated from the thymocyte-lymphocyte axis. The probable conclusion is that malignant cell types that never carry these antigens do not originate from the thymocyte-lymphocyte axis. Consequently, their observed absence from the myelomas that we have studied implies that normal plasma cells do not carry them either. This conclusion is strengthened by the finding that plaque-forming cells, both γG-producing and γM-producing, are insensitive to cytotoxic antisera belonging to any of these systems and so must lack these antigens. Therefore, either (*a*) the plasma cell does not belong to the thymocyte-lymphocyte axis but is derived from another type of lymphocyte (presumably from one lacking these antigens or perhaps not from a lymphocyte at all) or (*b*) differentiation to a plasma cell involves the lymphocyte in another metamorphosis of cell surface structure, added to that accompanying its differentiation from the stem cell, with all that this entails in the way of selective gene repression and derepression. Even granted a surface membrane turnover sufficiently rapid to transform the phenotype, the former interpretation seems more probable, i.e., that the plasma cell, whether or not derived from a cell with lymphocyte morphology, is not derived from a lymphocyte of the thymocyte-lymphocyte axis.

The most likely alternative parent for the plasma cell is a lymphocyte of the postulated bursa-lymphocyte axis in birds (28, 29), and of the hypothetical equivalent system in other animals (30). This suggests that bursal lymphocytes of birds will be found to have a set of differentiation antigens distinguishing them from thymic lymphocytes. Absence of the thymocyte-lymphocyte differ-

[3] In unpublished experiments with Dr. M. Mandel, we found that thoracic duct cells react like lymph node lymphocytes in tests with TL, θ, Ly-A, Ly-B, and MSLA antisera.

entiation antigens from plaque-forming cells of mice favors this possibility, and so does the presence of PC.1 alloantigen on plasma cells, which distinguishes them positively from thymocytes and from at least a large proportion of lymphocytes.

SUMMARY

A serological study of immunoglobulin-forming cells of the mouse, normal and malignant, shows that they lack all known surface differentiation antigens of the thymocyte-lymphocyte axis: TL, θ, Ly-A, Ly-B, and MSLA. Two systems of normal alloantigens are expressed on these cells, H-2 and a new system named PC.

The gene *Pca* (*Plasma cell antigen*) which specifies PC.1 alloantigen segregates as a mendelian dominant not closely linked with *H-2*. This cell surface antigen is absent from thymocytes, leukemias, and very probably from thymus-derived lymphocytes also; it is present on cells of the liver, kidney, brain, and lymph nodes as well as on hemolytic plaque-forming cells of the spleen, and on myelomas. So PC.1 is properly classified as a differentiation alloantigen. The strain distribution of PC.1 does not conform to that of any known immunoglobulin allotype or cell surface alloantigen previously described.

Thus the cell surface antigens of immunoglobulin-producing cells are clearly different from those of cells belonging to the thymocyte-lymphocyte axis. Each family of cells has distinctive alloantigens, and the two families share alloantigens of only one known system, H-2. This implies that either immunoglobulin-producing cells are not derived from thymic lymphocytes, or if they are, the program responsible for the transition must include extensive revision of cell surface structure.

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