

IMMUNOGLOBULIN AND OTHER SURFACE ANTIGENS OF
CELLS OF THE IMMUNE SYSTEM*

BY TOSHITADA TAKAHASHI, M.D., LLOYD J. OLD, M.D.,
K. ROBERT McINTIRE, M.D., AND EDWARD A. BOYSE, M.D.

(From the Division of Immunology, Sloan-Kettering Institute for Cancer Research, New York 10021; and the Laboratory of Biology, National Cancer Institute, National Institutes of Health, Bethesda, Maryland 20014)

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It is remarkable that of the six separate systems of surface antigens identified on thymocytes and lymphocytes of the mouse with cytotoxic antisera (1), all but one (H-2) are represented only, or mainly, on these cell types. Another antigen system with characteristically limited tissue representation has recently been recognized on plasma cells (2). We have referred to such discriminatory surface components as "differentiation antigens" (1).

Thus the antigenic constitution of the surfaces of immunocompetent cells, and by inference also the spectrum of genes used by these cells to build their surface membranes (1), is characteristic of this pathway of differentiation. Continuing our study of the antigenic composition of cells belonging to the immune system, we have examined the question "What immunoglobulin components can be identified as surface elements of immunocompetent cells of different types?" There is already a variety of evidence from other laboratories that Ig components are in fact detectable on such cells (see review ref. 3).

To do this we have studied the reactivity of a range of anti-Ig sera in tests with various immunocompetent cell populations, using the same cytotoxicity test system that has been used to identify all the other known surface antigens of immunocompetent cells referred to above. The Ig phenotypes of these cells were then further defined by testing the ability of various anti-Ig sera to interfere with their activity in a number of well-defined tests that measure functions of immunocompetent cells.

In discussion, we shall attempt to summarize present information on the surface phenotypes of different cells belonging to the various compartments of the immune system, including all the known differentiation antigens which serve to distinguish one such population from another, of which the Ig components represent one category.

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Materials and Methods

Mice.—These were obtained either from our colonies or from the Jackson Laboratory, Bar Harbor, Maine.

Myelomas and Other Tumors.—Three BALB ascites myelomas, S19 ($\kappa\gamma 2a$), S41 ($\kappa\alpha$), and S129 ($\kappa\gamma 1$), were kindly provided by Drs. R. Hyman and M. Cohn of the Salk Institute, San Diego, Calif. Other myelomas, leukemias, and sarcomas used in this study were described elsewhere (2).

Antisera.—Antisera against mouse Ig components were produced as described by Potter (4). Purified myeloma proteins and urinary proteins were used to immunize rabbits, appropriate absorptions being performed as necessary to obtain monospecific sera.

Cytotoxicity Tests.—Method of Gorer and O'Gorman (5) with modifications (6). Using medium 199 as diluent, tubes were set up containing 0.05 ml each of: (a) serial dilutions of antiserum, (b) guinea pig serum (GPS)¹ diluted 1/3 or 1/4 as the source of complement (C), and (c) cells (5×10^6 /ml). These were incubated at 37°C for 45 min. The proportion of dead cells was determined by adding 0.1 ml of a freshly prepared 0.16% trypan blue (to stain dead cells) to each tube and counting in a hemacytometer.

Each test included controls in which the cells were incubated with either GPS alone, or antiserum (1/4) alone; these never contained more than 10% stained cells and are therefore omitted from all tables and figures.

Detection of Ig Components on Hemolytic Plaque-Forming Cells (PFC).—The principle of the test is to determine whether treatment of spleen cell suspensions with anti-Ig + C reduces their plaque-forming capacity. It is expected that PFC carrying the relevant Ig component would be lysed by this procedure (see ref. 2).

Spleen cell suspensions were prepared from BALB mice injected intravenously with 0.1 ml of 20% (v/v) sheep erythrocytes (SE). Mice were immunized once for IgM PFC tests and twice for IgG tests. Tubes were set up containing 0.3 ml each of: (a) $2-6 \times 10^6$ spleen cells from these mice, (b) rabbit antiserum of the selected Ig specificity, or (control) normal rabbit serum (NRS), diluted 1/12, and (c) 1/4 dilution of GPS (C source); these were incubated for 45 min at 37°C.

The cells were then washed twice and resuspended in approximately 0.3 ml of diluent; 0.1 ml was used for each of three plates prepared as described previously (2). Medium 199 was used as diluent. IgM PFC and IgG PFC were developed by the methods of Jerne et al. (7) and Dresser and Wortis (8), respectively.

Detection of Ig Components and Alloantigens on Rosette-Forming Cells (RFC).—The principle is similar to that for detecting antigen on PFC (above). For detecting Ig on RFC, the cells were incubated with anti-Ig but without C (blocking of Ig sites apparently being sufficient to inhibit rosette formation, without actual lysis of RFC). For detecting surface alloantigens, C was included, as in the case of PFC (above). Cell suspensions were prepared from spleens of BALB mice injected intraperitoneally with 0.1 ml of 20% (v/v) SE.

I. *Detecting Ig components:* For detecting Ig components on the surface of RFC, 0.4 ml of a suspension containing 2×10^6 spleen cells was incubated for 1 hr with 0.4 ml of a 1/10

¹ *Abbreviations used in this paper:* anti-Ig, a rabbit antiserum to a mouse immunoglobulin component: either type specific (κ , λ) or class specific (μ , α , $\gamma 1$, $\gamma 2a$, $\gamma 2b$), in contrast to alloantisera against nonimmunoglobulin components of the cell surface; BALB, BALB/c; B cell, thymus-independent cell; C, complement; GPS, guinea pig serum; GVH, graft-versus-host; LNC, lymph node cells; MBLA, mouse-specific bone marrow-derived lymphocyte antigen; MSLA, mouse-specific lymphocyte antigen; NMS, normal mouse serum; NRS, normal rabbit serum; PFC, plaque-forming cells; RFC, rosette-forming cells; SE, sheep erythrocytes; T cell, thymus-derived cell.

dilution of antiserum of the selected Ig specificity or of NRS; this and all other procedures were carried out in the cold. The cells were then washed twice and resuspended in approximately 0.4 ml of medium 199 containing 5% fetal calf serum. The technique of suspension-centrifugation (9) was used to induce rosette formation. 1.6×10^7 SE were added to 2×10^6 treated spleen cells and centrifuged in a 10×75 mm tube at 100 g for 15 min. The pellet was incubated for an additional 30 min. The pellet was resuspended to 0.4 ml and approximately 15,000 spleen cells were scanned in a hemacytometer to determine the RFC/ 10^6 cells.

II. *Detecting alloantigens:* For detecting alloantigens on RFC, 0.4 ml each of (a) spleen cells, (b) alloantiserum or normal mouse serum (NMS), diluted 1/10, and (c) 1/15 diluted rabbit C absorbed with mouse erythrocytes were incubated for 45 min in a 37°C water bath. (Rabbit serum gives better complementation than GPS with most mouse alloantisera.) The cells were then washed twice at 4°C and tested for rosette formation as described above.

The use of cells exposed to NMS as the standard for expressing per cent suppression of RFC is dictated by the fact that NMS itself has a variable suppressive effect on RFC. This is under investigation, particularly in reference to the anti-thymocyte-lymphocyte autoantibodies that occur naturally in mouse serum (see ref. 10).

Test for Suppression of the Ig Phenotype of Lymph Node Cells (LNC) and Myeloma Cells Exposed to Anti-Ig Sera.—The procedure for modulation of TL antigens in vitro (11) was followed. 5×10^6 MOPC-70A ($\kappa\gamma 1$) myeloma cells or BALB LNC were suspended in 1 ml of medium 199 containing anti- κ , anti- $\gamma 1$, or NRS at a final dilution of 1/15. Suspensions were incubated for 10–60 min at 0°C or 37°C and shaken every 10 min to keep the cells suspended. After the selected period of incubation, the cells were immediately cooled in an ice bath, washed twice in the cold, and examined in the cytotoxicity test.

RESULTS

I. *Cytotoxicity Tests with Anti-H Chain and Anti-L Chain Sera on Thymocytes and LNC (Table I).*—22 class-specific and type-specific anti-Ig sera were tested against BALB thymocytes and LNC (Table I). 30–40% of LNC were sensitive to anti- μ and anti- κ . Other strains of mice were tested and all gave the same reactions as BALB. The proportion of LNC killed was not increased by combining anti- μ and anti- κ sera, suggesting that the majority of the susceptible cells carry both μ and κ specificities. (The anti- μ and anti- κ sera which were mixed together for these particular tests were selected on the basis that they were monospecific for μ and κ , respectively, without the necessity for prior absorption with immunoglobulin components, thus avoiding cross-neutralization by free μ and κ determinants.) Some of the class-specific or type-specific sera among the 22 tested were cytotoxic for thymocytes but this had no relation to Ig specificities (see II below).

II. *The Origin of the Cytotoxic Reaction on Thymocytes (Fig. 1).*—As illustrated in Fig. 1, the cytotoxicity of anti- μ and anti- κ sera against LNC was not absorbed by thymocytes but was absorbed by LNC (or with serum, or with the relevant isolated serum components; see V below). In contrast, the cytotoxicity of such antisera for thymocytes was completely absorbed by thymocytes and LNC, but not by whole serum. Thus the cytotoxic activity of Ig antisera against thymocytes is due to their contamination with anti-cellular antibody, occurring

naturally in the rabbit serum or induced unintentionally by immunization. (Thymocytes are characteristically much more sensitive than lymphocytes to cytolysis by natural heteroantibody.)

III. Cytotoxicity Tests with Anti- κ and Anti- μ on cells of Various Tissues

TABLE I
Cytotoxicity Tests with Anti-H Chain and Anti-L Chain Sera on BALB LNC*

	Dilution of antiserum								
	1/4	1/8	1/4	1/8	1/16	1/32	1/64	1/128	
	Thymocytes		Lymph node cells						
	% Cells dead								
Anti-H chain sera									
Anti- μ	<10	<10	31	31	30	31	12	<10	
Anti- α	<10	<10	<10	<10	<10	—	—	—	
Anti- γ 1	<10	<10	<10	<10	<10	—	—	—	
Anti- γ 2a	<10	<10	<10	<10	<10	—	—	—	
Anti- γ 2b	<10	<10	<10	<10	<10	—	—	—	
Anti-L chain sera									
Anti- κ	<10	<10	40	41	40	37	30	<10	
Anti- λ	<10	<10	12	15	<10	—	—	—	

* Sera selected for lack of cytotoxic activity for thymocytes, see Table III for the reactions of these sera with myelomas.

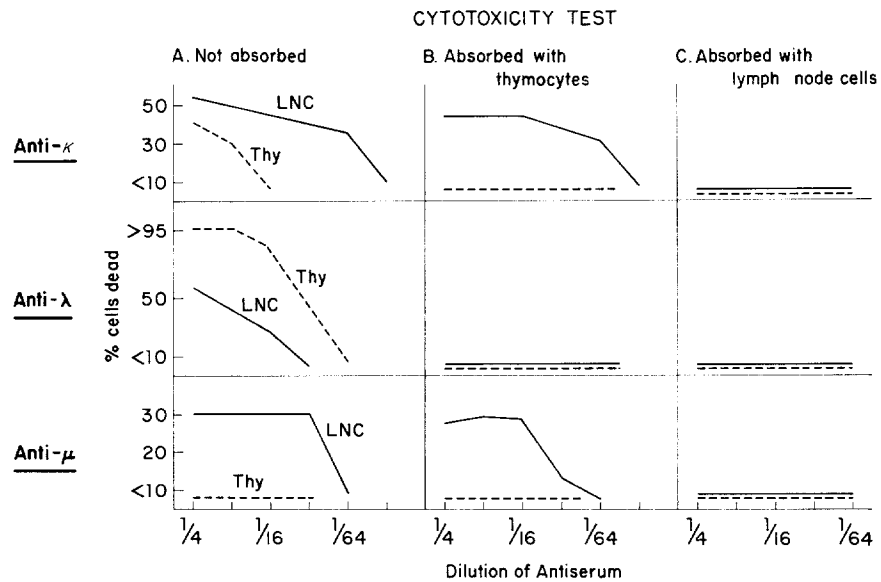


FIG. 1. Cytotoxicity tests with anti- κ , anti- λ , and anti- μ on lymph node cells (LNC) and thymocytes (Thy) (A) Effect of preabsorption with thymocytes (B) or LNC (C).

(Table II).—Lymphocytes separated from peripheral nodes, mesenteric nodes, blood, and peritoneal cavity were all in some proportion sensitive to anti- κ and anti- μ . Spleen contained the highest proportion of cells sensitive to anti- κ , and bone marrow the lowest. For each cell type the proportion of cells lysed by anti-

TABLE II
Cytotoxicity Tests with Anti- κ and Anti- μ Sera* on Cells of Various Tissues

Cell type (BALB)	Anti- κ			Anti- μ		
	1/4	1/16	1/64	1/4	1/16	1/64
	% Cells dead					
Thymocytes	< 10	< 10	< 10	< 10	< 10	< 10
Lymphocytes from						
Peripheral nodes	40	37	37	33	31	20
Mesenteric nodes	35	34	34	32	31	20
Blood	44	44	43	29	25	19
Peritoneal cavity	46	36	33	28	29	17
Spleen cells	55	59	38	36	30	21
Bone marrow cells	28	26	18	25	24	25

* Results not affected by preabsorption with thymocytes.

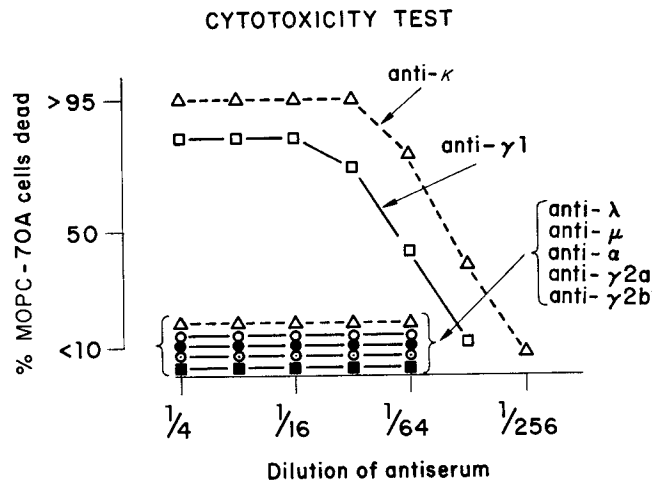


FIG. 2. Cytotoxicity tests with anti- $\gamma 1$ and anti- κ on MOPC-70A myeloma cells.

κ was somewhat higher than that lysed by anti- μ ; therefore particularly in the case of spleen, where the discrepancy was considerable, it seems likely that some κ^+ cells carry heavy chains other than μ .

IV. Cytotoxicity Tests with Anti-H Chain and Anti-L Chain Sera on Myeloma Cells (Table III and Fig. 2).—Three transplantable ascites myelomas were tested with the full range of antisera available to us (Table III). One of these,

MOPC-70A ($\kappa\gamma 1$), was sensitive to anti- κ and anti- $\gamma 1$. The other two, MOPC-104E ($\lambda\mu$) and MPC-67 ($\kappa\alpha$), were insensitive to all other Ig antisera. Five more ascites myelomas S129 ($\kappa\gamma 1$), S41 ($\kappa\alpha$), S19 ($\kappa\gamma 2a$), Adj-PC5 ($\kappa\gamma 2a$), and MPC-11 ($\kappa\gamma 2b$) were tested with anti- κ and appropriate anti-H chain sera;

TABLE III

Cytotoxicity Tests with Anti-H Chain and Anti-L Chain Sera on Myeloma Cells: Summary*

	MOPC-70A ($\kappa\gamma 1$)	MOPC-104E ($\lambda\mu$)	MPC-67 ($\kappa\alpha$)
Anti-H chain sera			
Anti- μ	-†	-	-
Anti- α	-	-	-
Anti- $\gamma 1$	+§	-	-
Anti- $\gamma 2a$	-	-	-
Anti- $\gamma 2b$	-	-	-
Anti-L chain sera			
Anti- κ	+	-	-
Anti- λ	-	-	-

* See Table I for the reactions of these sera with thymocytes and LNC.

† -, no cytotoxic reaction.

§ +, positive cytotoxic reaction, titer 1/64, see Fig. 2.

TABLE IV

Specificity of Cytotoxic Reactions of Anti-H Chain and Anti-L Chain Sera Confirmed by Neutralization with Ig Components

Antiserum	Preabsorption with			
	None	NMS	κ -chain	λ -chain
	% LNC dead			
Anti- μ	31	<10	27	35
Anti- κ	37	<10	<10	38
	% MOPC-70A cells dead			
Anti- $\gamma 1$	65	<10	60	62
Anti- κ	90	<10	<10	89

Method: 0.05 ml antiserum (diluted 1/8) incubated with 0.05 ml (a) NMS (1/10), (b) κ -chains (0.1 mg/ml), or (c) λ -chain (0.1 mg/ml) for 45 min at room temperature followed by 45 min in the cold and then titrated against LNC or MOPC-70A.

once again only the $\kappa\gamma 1$ -producing myeloma (S129) showed sensitivity to the appropriate anti-Ig sera in the cytotoxicity test (see Discussion).

A titration of anti- κ and anti- $\gamma 1$ against MOPC-70A is shown in Fig. 2. 20 leukemias of different types and from various mouse strains were tested with anti- κ , with negative results in all cases.

V. Confirmation of the Specificity of the Cytotoxic Reactions of Anti- μ , Anti- $\gamma 1$,

and Anti- κ Sera against LNC and Myeloma Cells (Table IV).—The cytotoxicity of anti- μ for LNC, and of anti- γ 1 for MOPC-70A cells, was abolished by preincubation of these antisera with NMS but not with κ -chains or λ -chains. Similarly the cytotoxic activity of anti- κ sera for LNC and MOPC-70A cells was abolished by incubating the antiserum with NMS or with κ -chains, but not with λ -chains.

TABLE V

Tests for Ig Components on Hemolytic Plaque-Forming Cells (PFC); Effect of Preincubating PFC with Anti-H and Anti-L Chain Sera

BALB spleen cells (PFC) preincubated with* (and complement)	Plaques: IgM type				Summary of results: degree of inhibition	Plaques: IgG type				Sum- mary of re- sults: degree of in- hibition
	Exp. 1		Exp. 2			Exp. 1		Exp. 2		
	Σ †	%§	Σ	%		Σ	%	Σ	%	
NRS	150	100	118	100		232	100	213	100	
Anti-H chain										
Anti- μ	56	37	59	50	+++	222	96	202	95	—
Anti- α	134	89	109	92	—	246	106	213	100	—
Anti- γ 1	152	102	108	91	—	235	101	206	97	—
Anti- γ 2a	137	91	121	103	—	210	91	198	93	—
Anti- γ 2b	132	88	115	97	—	214	92	187	88	—
Anti-L chain										
Anti- κ	74	49	58	49	+++	213	92	189	89	—
Anti- λ	140	93	120	102	—	197	85	211	99	—

* Dilution of all antisera 1/12; conditions of preincubation of spleen cells as for cytotoxicity tests.

† Total plaques in three similar plates.

§ PFC/(PFC with NRS) \times 100.

|| % inhibition: — <15, + 16-30, ++ 31-50, +++ 51-70, ++++ 71-100. (Based on the two results to the left.)

VI. Tests for Ig Components on Hemolytic Plaque-Forming Cells (Table V).—Spleen cells from mice immunized against SE were incubated with anti-H chain or anti-L chain sera, together with C. Their plaque-forming capacity was then tested directly for IgM PFC and indirectly (i.e. with added anti-mouse Ig) for IgG PFC.

IgM PFC cells were suppressed by anti- μ and also by anti- κ (50% or more reduction of plaques in both cases). The controls for these experiments included prior neutralization of the anti- μ and anti- κ reagents with NMS, and substitution of heat-inactivated C, both of which abolished the suppression of IgM PFC.

In contrast, no significant demonstrable suppression of IgG PFC was ob-

tained with any of the H chain or L chain antisera. We considered the possibility that some IgG PFC might indeed be carrying class-specific determinants on their surfaces but that for each particular specificity the proportion of cells was too low for their loss to be recognizable as a lowering of the number of plaques. To cover this possibility a number of tests were performed in which the plaque reaction was developed with class-specific antisera instead of the

TABLE VI
Tests for Inhibition of Rosette-Forming Cells (RFC) by
Anti-H Chain and Anti-L Chain Sera

Spleen cells (RFC) preincubated* with	Day 4‡				Day 9‡				Summary¶ of results: degree of inhibition
	Exp. 1		Exp. 2		Exp. 1		Exp. 2		
	RFC§	%	RFC	%	RFC	%	RFC	%	
NRS	397	100	327	100	426	100	624	100	—
Anti-H chain									
Anti- μ	159	40	111	33	198	46	366	58	+++
Anti- α	343	86	274	83	306	71	561	89	+
Anti- γ 1	350	88	267	82	307	71	538	86	+
Anti- γ 2a	287	72	287	87	337	79	389	62	+
Anti- γ 2b	314	79	251	76	297	69	393	63	+
Anti-L chain									
Anti- κ	20	5	26	8	53	12	53	9	++++
Anti- λ	429	109	343	105	521	122	663	106	—

* 0.4 ml spleen cell suspension (5×10^6 /ml) incubated with 1/10 antiserum for 60 min at 4°C. The cells were then washed and tested for rosette formation.

‡ BALB mice were immunized with 0.1 ml of 20% (v/v) SE intraperitoneally. Tests for RFC were performed 4 or 9 days later.

§ Average of two duplicate samples. Approximately 15,000 spleen cells scanned in each sample to determine the RFC/ 10^6 cells.

|| RFC/(RFC with NRS) \times 100.

¶ See footnote ||, Table V.

usual polyspecific anti-Ig. The number of plaques obtained was considerably lower in each case, as expected, but again preincubation with class-specific antisera in the presence of C produced no detectable suppression of the respective IgG PFC. There remains the possibility that unidentified H chain determinants are expressed on these cells, but the failure of anti-L chain sera to suppress IgG PFC is against this; no matter what heavy chain determinants are expressed on these cells, it is to be expected that they should have either κ - or λ -chains. We conclude that no Ig components are demonstrable by these methods on the surface of IgG PFC.

The results in this section parallel closely those in section I above, where it was seen that the cytotoxicity test recognizes μ and κ on a proportion of lymphoid cells, but does not recognize cells bearing other Ig components. This does not mean they are not there, in fact $\gamma 1$ is demonstrable on two myelomas, but more likely that the amount on LNC and bone marrow cells is too small to give rise to a significantly positive cytotoxicity test.

VII. *Ig Components and Alloantigens on the Surface of Rosette-Forming Cells (Tables VI and VII).*—The major Ig determinants on the surface of RFC are μ and κ ; anti- μ inhibited rosette formation by 40–60% and anti- κ by approxi-

TABLE VII

Tests for H-2, θ , Ly-A, Ly-B, Ly-C, and PC.1 Alloantigens on Rosette-Forming Cells (RFC); Effects of Pre-incubating RFC with the Respective Alloantisera

Spleen cells (RFC) preincubated* with	Day 4†				Day 7‡				Summary of results: degree of inhibition
	Exp. 1		Exp. 2		Exp. 1		Exp. 2		
	RFC§‡	%§‡	RFC	%	RFC	%	RFC	%	
NMS	675	100	667	100	539	100	844	100	—
Anti- θ -C3H¶	750	111	641	96	525	97	859	102	—
Anti-Ly-A.2	645	96	587	88	654	119	737	87	—
Anti-Ly-B.2	545	81	782	117	539	100	781	92	—
Anti-Ly-C.2	539	80	647	97	600	111	683	80	—
Anti-PC.1	480	71	516	77	378	70	639	76	+
Anti-H-2 ^a	27	4	20	3	20	4	53	6	++++

* Dilution of serum 1/10, C, 1/8 rabbit serum preabsorbed with mouse thymocytes and erythrocytes, conditions of preincubation of spleen cells as for cytotoxicity test.

† A strain mice were immunized with 0.1 ml 20% (v/v) SE intraperitoneally and tests for RFC were performed 4 or 7 days later.

§ See footnotes §, || Table VI.

|| See footnote ||, Table V.

¶ Prepared by immunization of A/ θ -AKR congenic mice with A strain (θ -C3H) cells.

mately 90%. Lesser reductions were observed with all the other anti-Ig sera with the exception of anti- λ (Table VI). These reductions are significant and indicate that heavy chains other than μ are also present on RFC.

Incubation of spleen cells with H-2 antiserum and C virtually abolished all RFC. Anti-PC.1 serum (2) reduced RFC by 25–30%. No consistent reduction was produced by antisera belonging to the θ , Ly-A, Ly-B, or Ly-C systems of thymocyte-lymphocyte antigens (1).

The usual θ antisera, C3H anti-AKR and AKR anti-C3H (12), reduced RFC by 30–40%, as others have reported (13, 14). This is not due to anti- θ , however, for θ antisera prepared in θ -congenic mice, with anti- θ titers as high as or higher than the C3H anti-AKR and AKR anti-C3H, gave no reduction of

CYTOTOXICITY TEST

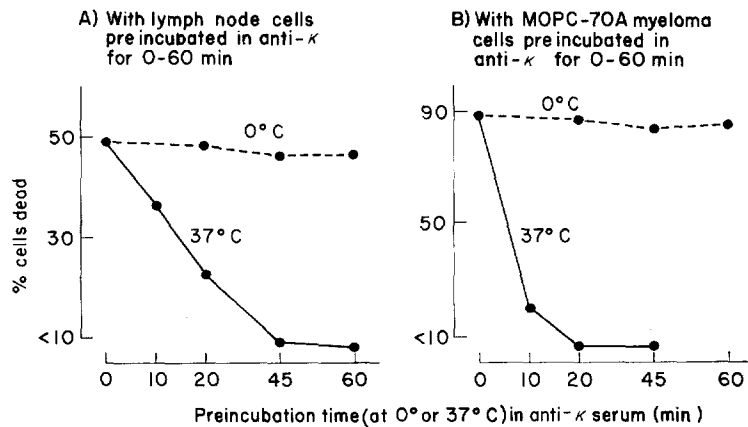


FIG. 3. Suppression of κ^+ phenotype of LNC and myeloma cells by exposure to anti- κ in the absence of complement. LNC (A) or MOPC-70A myeloma cells (B) were incubated with 1/15 anti- κ for a period of 10-60 min (abscissa) at 0°C or 37°C and then subjected to the standard cytotoxicity test with 1/15 anti- κ (ordinate = per cent cells lysed). (Curves for sensitivity to added C alone, not shown, were similar to those in this figure. Control cells preincubated in NRS at 37°C, instead of anti- κ , showed no loss of sensitivity to anti- κ .)

CYTOTOXICITY TEST

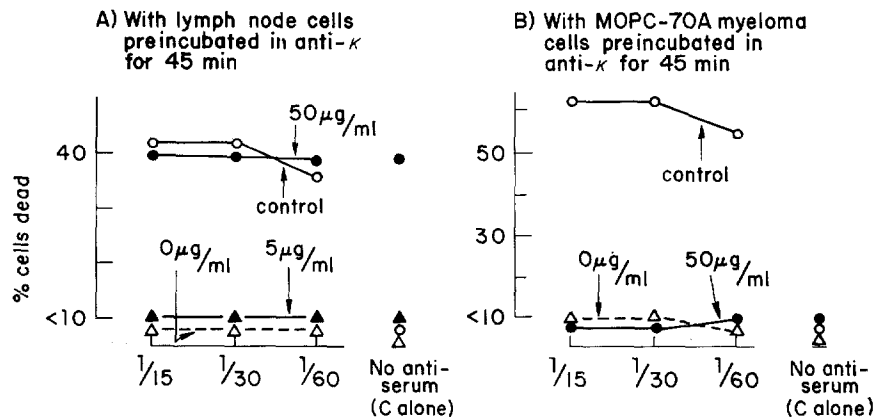
Dilution of anti- κ in cytotoxicity test

FIG. 4. Effect of actinomycin D on the $\kappa^+ \rightarrow \kappa^-$ surface-phenotype change induced in LNC (A) and MOPC-70A myeloma cells (B). *Step 1*: LNC or MOPC-70A myeloma cells incubated in actinomycin D (5 or 50 $\mu\text{g/ml}$) for 60 min at 37°C, then were washed twice in the cold. *Step 2*: Further incubating in 1/15 anti- κ or NRS (control) for 45 min at 37°C; cells then washed twice in the cold. *Step 3*: This figure; cytotoxicity tests with anti- κ .

Interpretation: actinomycin D (50 $\mu\text{g/ml}$) inhibited the $\kappa^+ \rightarrow \kappa^-$ conversion of LNC (but not of MOPC-70A myeloma cells) induced by exposure to anti- κ .

RFC. This must therefore be an antibody of another specificity which reacts with RFC. We find that this unknown antibody does not belong to any of the Ly specificities (15) that are found in low titer in C3H anti-AKR and AKR anti-C3H sera.

VIII. *Suppression of the Surface Ig Phenotype of LNC and Myeloma Cells Exposed to Anti-Ig Sera (Fig. 3-5).*—As described above, a proportion of LNC and MOPC-70A cells are sensitive to lysis by anti- κ in the presence of C. However, when these cells are incubated with anti- κ in the absence of C their sensi-

CYTOTOXICITY TEST WITH CELLS PREINCUBATED IN ANTI- $\gamma 1$ OR ANTI- κ

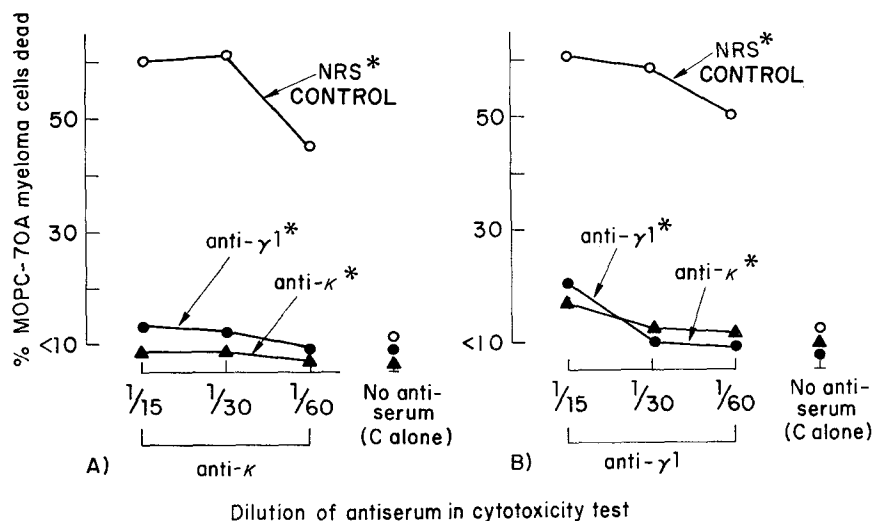


FIG. 5. Loss of $\kappa^+ \gamma 1^+$ phenotype of myeloma cells incubated in either anti- κ or anti- $\gamma 1$ in the absence of complement. *Step 1:* MOPC-70A myeloma cells incubated with *1/15 NRS, anti- κ , or anti- $\gamma 1$ for 60 min at 37°C, then washed twice in the cold. *Step 2:* This figure; cytotoxicity tests with anti- κ (A), or anti- $\gamma 1$ (B).

tivity to lysis by C decreases progressively; finally they become totally insensitive to added C and are no longer susceptible to lysis by the addition of fresh anti- κ and C (Fig. 3). Thus the apparent Ig surface phenotype changes from κ^+ to κ^- . This change of phenotype did not occur if the cells were incubated at 0°C, and in the case of LNC, phenotypic suppression by anti- κ could also be inhibited by actinomycin D, although this was not possible with MOPC-70A cells (Fig. 4). In a similar manner, exposure of MOPC-70A cells to anti- $\gamma 1$ serum altered the phenotype from $\gamma 1^+$ to $\gamma 1^-$ (Fig. 5).

The presence of both κ and $\gamma 1$ on MOPC-70A cells enabled us to determine whether phenotypic suppression of one of them would entail phenotypic suppression of the other. It appears that this is the case (Fig. 5). MOPC-70A cells

incubated with anti- $\gamma 1$ became insensitive to both anti- κ and anti- $\gamma 1$, and MOPC-70A incubated with anti- κ also became insensitive to both anti- $\gamma 1$ and anti- κ .

DISCUSSION

Ig and Other Surface Markers on Cells of the Immune System.—Table VIII summarizes what is known of the surface antigens expressed on cells of the immune system. These data accord with the prevailing view that lymphoid cells belong to two major classes, the thymus-derived (T) cell and the thymus-independent (B) cell originating from bone marrow and thought to be the equivalent of the bursal cell of avian species (16, 17). The surface markers of the T cell are the θ -Ly-MSLA-TL antigens (1), and for the B cell the PC-MBLA antigens (2, 18).

PC evidently is not a good marker for the immature B cell, for it seems to occur rather late in differentiation (see below), at a time when the B cell is about to become a plasma cell and to secrete Ig. These two sets of markers (T versus B) appear to be mutually exclusive. We have found no evidence of a normal or malignant cell carrying both of them.

What is the origin of cells bearing demonstrable Ig on their surface? Those bearing PC.1 as well as Ig are clearly B cells; in this category are a proportion of IgM PFC and of RFC (and $\kappa\gamma 1$ myelomas). Do T cells also carry Ig markers at some time in their history? If so, one might hope to find some cells bearing both thymocyte markers and Ig. It has been reported that some rosette-forming cells are θ^+ (13, 14), but we find that monospecific θ antisera prepared in congenic mice do not react with RFC; suppression of RFC by the usual anti- θ sera (12) is due to an unidentified antibody; this may be recognizing a T cell marker, but that has yet to be proved. If indeed some RFC are T cells, then the T cell must acquire the RFC property late in its history, at a time when θ and Ly are reduced to nondetectable levels. Thus, despite the apparent necessity for surface Ig receptors on T cells to account for antigen-recognition, we have not succeeded in finding a T cell surface marker on any Ig $^+$ cell. But it would certainly be unjustified to conclude that the Ig $^+$ surface phenotype is confined to B cells.

Given the facts about the B and T cell surface markers, what can be said about the origin of the various cell populations examined? The easiest to assess are the clonal populations in Table VIII: (a) the lymphatic leukemia cells, which are θ^+ Ly $^+$ PC $^-$ Ig $^-$ (T cell surface phenotype) and (b) the myeloma cells which are θ^- Ly $^-$ PC $^+$ Ig $^+$ or $^-$ (B cell surface phenotype). Among normal cells, thymocytes (θ^+ Ly $^+$ PC $^-$ Ig $^-$) come closest to being a similarly homogeneous population. Cell suspensions from other organs contain various proportions of θ^+ and κ^+ cells (bone marrow 30% κ^+ , none θ^+ ; spleen 50% κ^+ , 30% θ^+ ; thoracic duct 15% κ^+ , 80% θ^+). Although the minimum proportion of T cells is given by the percentage of cells showing the θ phenotype, the proportion of B cell is

TABLE VIII
Surface Antigens of Immunocompetent Cells: Summary Table*

Cell surface antigens	Serological tests in vitro										Tests in vivo				
	Cytotoxicity test										GVH§	Adoptive transfer of antibody formation			
	Thymo- cytes	Thoracic duct cells	Lymph node cells	Spleen cells	Bone marrow cells	Lym- phocytic leukemia cells	Myelomas		Rosette formation	Plaque formation		1°¶	2°	vs. <i>Brucella abortus</i>	
						κγ1	Others	RFC (day 4)	IgM	IgG	1°				2°
θ	+ (>95)	+ (80)	+ (50)	+ (30)	-	+	-	-	-	-	-	+	+	+	-
Ly-A, B, C	+ (>95)	+ (30-60)	+ (20-50)	+ (10-50)	-	+	-	-	-	-	-	-	-	-	-
PC	-	-	-	-	-	-	+	+	+	+	+	+	+	+	-
κ	-	+ (15)	+ (40)	+ (50)	+ (30)	-	+	+	+	+	+	+	+	+	-
μ	-	+ (10)	+ (30)	+ (40)	+ (20)	-	-	-	+	+	+	+	+	+	-
Other Ig compo- nents	-	-	-	-	-	-	+	+	+	+	+	+	+	+	-

* This table summarizes data from the present study and from previous publications from this laboratory (see refs. 2, 15, 21, 22).

† Percentage of dead cells indicated by direct cytotoxicity tests. — = < 10.

§ Based on Simonsen's spleen assay (27); adult BALB spleen cells were incubated at 37°C for 45 min with (a) anti-θ-C3H (congenetic) (diluted 1/20), (b) anti-κ (diluted 1/12), (c) NMS (diluted 1/20), or (d) NRS (diluted 1/12). Cells were washed twice and injected i.v. (2 × 10⁶/recipient) into newborn (BALB × C57BL/6) F₁ mice.

|| Lymphocytes from peritoneal cavity and blood also gave similar results.

¶ 1° = primary response; 2° = secondary response.

** Anti-whole Ig serum (containing anti-κ) was used instead of monospecific anti-κ.

uncertain, because we cannot be sure that the κ surface phenotype absolutely distinguishes B cells from T cells.

What are the relative contributions of B and T cells to the various cell populations concerned in tests of function *in vitro* and *in vivo*? (a) Some RFC are B cells (PC^+); the contribution of T cells to this population is uncertain (as discussed above). (b) Most or all PFC have the B phenotype (PC^+). Possibly the B-type RFC which carry surface Ig mature into PFC. One interpretation is that the B cell mediating rosette formation is at an early phase of differentiation during which it expresses surface Ig; as it matures to the secretory phase, the amount of surface Ig decreases and PC increases. This may signify a remodeling of the B cell surface as it undergoes maturation to an Ig-secreting end cell, perhaps the counterpart of the profound transition in alloantigen representation which the thymocyte experiences in the course of peripheralization (1). (c) The cell population which has the potential to initiate graft-*versus*-host (GVH) reactions has the T phenotype $\theta^+\kappa^-$ (evidently the majority of such cells do not have detectible amounts of surface Ig). (d) Antibody formation in mice involves in some instances a thymus-dependent function, exemplified by the response to SE (19, 20), and in others a thymus-independent function, exemplified by the response to *Brucella* antigens (21). The surface phenotypes are concordant (22) since adoptive immunization of the former type was antagonized by θ antiserum (implicating the T cell) whereas the latter was not (Table VIII) (21).

The failure of PC antiserum to influence adoptive transfer of primary or secondary antibody formation is paradoxical, because antibody-producing cells are PC^+ (2). The most likely explanations are that: (a) PC^+ cells may be relatively resistant to radiation, enabling them to survive after the prospective recipient has been irradiated in preparation for the test of adoptive immunization (the donor PC^+ cells would thus be superfluous, and their destruction by PC antiserum immaterial); or (b) at the time of transfer the cells responsible may be at an early stage of maturation at which we suspect the cell with antibody-secretory potential is PC^- . (For a discussion of evidence favoring the latter interpretation see ref. 22.)

With regard to myelomas it is somewhat surprising that the only surface immunoglobulin type found was $\kappa\gamma 1$ rather than $\kappa\mu$ which is the type demonstrable on lymphocytes and IgM PFC. A total of four $\kappa\gamma 1$ -producing myelomas (two in this report and two tested by Princler, G. L., and K. R. McIntire, unpublished) have been examined; all were sensitive to anti- κ and anti- $\gamma 1$. Six myelomas of other classes (1μ , 2α , $2\gamma 2a$, $1\gamma 2b$) have not been sensitive to anti-Ig sera in cytotoxicity tests.

Thus we can broadly recognize three Ig surface phenotypes: (a) surface Ig only, no demonstrable secretion, e.g. the $\kappa^+\mu^+$ lymphoblastic human cell line (Daudi, ref. 23) and presumably also the $\kappa^+\mu^+$ lymphocyte; (b) secretory Ig

only, no demonstrable surface Ig, e.g. most mouse myelomas and IgG PFC; and (c) combined surface and secretory type, e.g. $\kappa\gamma 1$ myelomas and the IgM PFC.

Antibody-Induced Suppression of the Ig Phenotype: Antigenic Modulation?—The apparent loss of Ig from the surfaces of cells incubated in anti-Ig sera has several features in common with antigenic modulation described in the TL system of antigens (11). Both phenomena are temperature dependent, both can be suppressed by actinomycin D, and in both instances the loss may include specificities not represented in the antiserum responsible for the suppression. In the TL system a simple blocking mechanism has been ruled out, and we are left with the possibilities that the modulated antigen has been (a) exfoliated from the cell, (b) ingested by the cell, or (c) combined with antibody in a manner leading to conformational changes at the cell surface which ultimately prevent the effective utilization of complement.

The fact that phenotypic suppression resembling antigenic modulation is now seen to affect cell surface Ig determinants adds weight to the suggestion (24) that antigenic modulation of TL antigens may be a model for "allotype suppression" (the suppression of an Ig allotype in the serum of an animal treated with antibody to that allotype) (25). The union of anti-Ig with cell surface Ig is an event that involves the same surface Ig molecule that would normally engage antigen. Either stimulus can initiate blastic transformation of the lymphocyte (26). In both cases we surmise that this process begins with suppression of the Ig surface phenotype, as observed in our experiment, and in the case of the B cell ends with conversion into the Ig-secretory phenotype.

SUMMARY

Immunoglobulins (Ig) on cells of the immune system: The cytotoxicity test, with class-specific and type-specific anti-Ig sera, identifies κ and μ determinants on mouse lymphocytes. The proportion of κ^+ cells is characteristic for each source of cells: 30% of bone marrow cells, 40% of cells from peripheral lymph nodes, 45% of lymphocytes from peripheral blood or peritoneal cavity, and 50% of spleen cells. No Ig was demonstrable on thymocytes or on leukemia cells (most of which arise from thymus-derived [T] cells). Cytotoxicity tests were performed on various myelomas secreting different Ig; the only positive reactions were given by $\kappa\gamma 1$ myelomas (all four $\kappa\gamma 1$ myelomas tested were sensitive to both anti- κ and anti- $\gamma 1$). Hemolytic plaque-forming cells (PFC) of IgG type had no demonstrable surface Ig, but a proportion of IgM PFC were $\kappa^+\mu^+$. Virtually all rosette-forming cells (RFC) have surface Ig, more than 90% of them being inhibited by anti- κ , 50% by anti- μ , and 10–30% by antisera to other heavy chains. Anti- λ sera gave no positive reactions with any cell type, which is in keeping with the low level of this light chain in mouse serum.

Ig and other differentiation antigens as markers for T and B cells: Thymocytes are hallmarked by the alloantigens TL, θ , and the Ly series, and it is generally held that extrathymic lymphoid cells that bear them are derived from thymocytes. There is one alloantigen marker for the thymus-independent (B) cell, and that is PC, which appears late in differentiation. (The mouse-specific lymphocyte (MSLA) and mouse-specific bone marrow-derived lymphocyte (MBLA) antigens recognized by heteroantisera, not used in the present study, are other candidates for T and B cell markers.) Making use of antisera to these surface antigens to inhibit the function of cells that carry them, we find the following:

Approximately 30% of RFC, 60% of IgM PFC, and 90% of IgG are PC⁺ and so are identified as B cells. No T markers were demonstrable on these cell populations. Thus if T cells do become RFC or PFC they presumably lose their T surface markers in the process (cf. the quantitative reduction of T markers accompanying the thymocyte \rightarrow lymphocyte transition).

Cells that have the potential to initiate graft-*versus*-host (GVH) reactions have the T cell surface phenotype θ^+ Ig⁻.

Adoptive transfer of thymus-dependent antibody-forming capacity (response to sheep erythrocytes) required θ^+ cells but transfer of a thymus-independent immune response to *Brucella* antigen did not. Cells with surface Ig were involved in both types of adoptive transfers.

Thus the presently available T markers do not provide evidence for T cells carrying surface Ig.

Suppression of the Ig phenotype by antibody: antigenic modulation? A phenotypic change from Ig⁺ to Ig⁻ occurs when Ig⁺ lymphocytes or myeloma cells are incubated with anti-Ig sera in vitro in the absence of complement (C). As with antigenic modulation in the TL system, which it resembles, this phenomenon is temperature dependent and in the case of lymph node cells (LNC) can be inhibited by high doses of actinomycin D.

Note Added in Proof.—Subsequent tests show RFC to be MSLA⁻ and a high population of them MSPCA⁺. The antigen MSPCA (mouse-specific plasma cell antigen) identified by certain absorbed rabbit anti-mouse myeloma sera is a differentiation antigen of Ig-secreting cells, and is absent from T cells (manuscript in preparation).

BIBLIOGRAPHY

1. Boyse, E. A., and L. J. Old. 1969. Some aspects of normal and abnormal cell surface genetics. *Annu. Rev. Genet.* **3**:269.
2. Takahashi, T., L. J. Old, E. A. Boyse. 1970. Surface alloantigens of plasma cells. *J. Exp. Med.* **131**:1325.
3. Greaves, M. F. 1970. Biological effects of anti-immunoglobulins: evidence for immunoglobulin receptors on "T" and "B" lymphocytes. *Transplant. Rev.* **5**:45.
4. Potter, M. 1967. The plasma cell tumors and myeloma proteins of mice. *Methods Cancer Res.* **2**:105.

5. Gorer, P. A., and P. O'Gorman. 1956. The cytotoxic activity of isoantibodies in mice. *Transplant. Bull.* **3**:142.
6. Boyse, E. A., L. J. Old, and I. Chouroulinkov. 1964. Cytotoxic test for demonstration of mouse antibody. *Methods Med. Res.* **10**:39.
7. Jerne, N. K., A. A. Nordin, and C. Henry. 1963. The agar plaque technique for recognizing the antibody producing cells. *In Cell Bound Antibodies*. B. Amos and H. Koprowski, editors. The Wistar Institute Press, Philadelphia, Pa. 109.
8. Dresser, D. W., and H. H. Wortis. 1965. Use of an antiglobulin serum to detect cells producing antibody with low haemolytic efficiency. *Nature (London)*. **208**: 859.
9. Edwards, G. E., R. G. Miller, and R. A. Phillips. 1970. Differentiation of rosette forming cells from myeloid stem cells. *J. Immunol.* **105**:719.
10. Shirai, T., and R. C. Mellors. 1971. Natural thymocyte toxic autoantibody and reactive antigen in New Zealand Black mice and mice of other strains *Proc. Nat. Acad. Sci. U.S.A.* **68**:1412.
11. Old, L. J., E. Stockert, E. A. Boyse, and J. H. Kim. 1968. Antigenic modulation. Loss of TL antigens from cells exposed to TL antibody. Study of the phenomenon in vitro. *J. Exp. Med.* **127**:523.
12. Reif, A. E., and J. M. V. Allen. 1964. The AKR thymic antigen and its distribution in leukemias and nervous tissues. *J. Exp. Med.* **120**:413.
13. Schlesinger, M. 1970. Anti- θ antibodies for detecting thymus-dependent lymphocytes in the immune response of mice to SRBC. *Nature (London)*. **226**:1256.
14. Greaves, M. F., and E. Möller. 1971. Studies on antigen binding cells. I. Origin of reactive cells. *Cell. Immunol.* **1**:372.
15. Boyse, E. A., M. Miyazawa, T. Aoki, and L. J. Old. 1968. Ly-A and Ly-B. Two systems of lymphocyte iso-antigens in the mouse. *Proc. Roy. Soc. Ser. B Biol. Sci.* **170**:175.
16. Warner, N. L., and A. Szenberg. 1964. Immunologic studies on hormonally bursctomized and surgically thymectomized chickens: dissociation of immunologic responsiveness. *In Thymus in Immunobiology*. R. A. Good and A. E. Gabrielsen, editors. Hoeber-Harper, New York. 395.
17. Cooper, M. D., A. E. Gabrielsen, and R. A. Good. 1967. Role of the thymus and other central lymphoid tissues in immunological disease. *Annu. Rev. Med.* **18**: 113.
18. Raff, M. C., S. Nase, and N. A. Mitchison. 1971. Mouse specific bone marrow derived lymphocyte antigen as a marker for thymus-independent lymphocytes. *Nature (London)*. **230**:50.
19. Claman, H. N., E. A. Chaperon, and R. F. Triplett. 1966. Thymus-marrow cell combinations—synergism in antibody production. *Proc. Soc. Exp. Biol. Med.* **122**:1167.
20. Mitchell, G. F., and J. F. A. P. Miller. 1968. Cell to cell interaction in the immune response. II. The source of hemolysis-forming cells in irradiated mice given bone marrow and thymus or thoracic duct lymphocytes. *J. Exp. Med.* **128**:821.
21. Thorbecke, G. J., T. Takahashi, and W. P. McArthur. 1971. Surface antigens of immunocompetent cells. *In Morphological and Functional Aspects of Immunity*.

- K. Lindahl-Kiessling, G. Alm, and M. G. Hanna, Jr., editors. Plenum Publishing Corporation, New York. 467.
22. Takahashi, T., E. A. Carswell, and G. J. Thorbecke. 1970. Surface antigens of immunocompetent cells. I. Effect of θ and PC.1 alloantisera on the ability of spleen cells to transfer immune responses. *J. Exp. Med.* **132**:1181.
 23. Klein, E., J. Eskeland, M. Inoue, R. Strom, and B. Johanson. 1970. Surface immunoglobulin moieties on lymphoid cells. *Exp. Cell Res.* **62**:133.
 24. Herzenberg, L. A., E. B. Jacobson, L. A. Herzenberg, and R. Riblet. Chronic allotype suppression in mice: an active regulatory process. *Ann. N.Y. Acad. Sci.* In press.
 25. Herzenberg, L. A., H. D. McDevitt, and L. A. Herzenberg. 1968. Genetics of antibodies. *Annu. Rev. Genetics.* **2**:209.
 26. Sell, S., and P. G. H. Gell. 1965. Studies on rabbit lymphocytes in vitro. I. Stimulation of blast transformation with an antiallotype serum. *J. Exp. Med.* **122**: 423.
 27. Simonsen, M. 1962. Graft-versus-host reactions. Their natural history and applicability as tools of research. *Progr. Allergy.* **6**:349.