Immunology, 1971, **20**, 1087.

Specificity of Rabbit Antisera against Mouse Leukaemias *in vitro*

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(*Received* 26*th August* 1970)

Summary. Rabbit antisera were prepared against mouse leukaemias from several inbred strains, and against a mixture of these leukaemias. The potencies of these antisera were tested against each of the different leukaemias, and against certain normal cells and tissues.

As determined by immune cytolysis, the potencies of antisera were strongest against the homologous leukaemias. The potencies against unrelated leukaemias were generally within 50 per cent of those against the homologous leukaemias, with some exceptions. Antisera prepared by injection of a mixture of the leukaemia cells showed remarkably high potencies against all leukaemias tested.

As measured by C fixation, the same antisera showed less specificity for the homologous leukaemias. The potencies of antisera for C fixation on a variety of normal tissues was quite strong, and decreased approximately in the following order: testis, kidney, liver, lung, brain, muscle, erythrocytes. For antiserum prepared against DBA/2 leukaemia L1210, the C fixation reaction against normal lymph node lymphocytes was about 20 per cent as strong as the reaction against the homologous leukaemia L1210. A hypothesis concerning the mechanism of immune cytolysis is presented to explain these results. Exhaustive absorption of anti-L1210 sera with normal DBA/2 tissues produced only a slight increase in anti-leukaemia specificity.

INTRODUCTION

A number of studies on the effect of heterologous antisera on experimental leukaemias have shown mild but significant therapeutic benefits (Mohos and Kidd, 1957; Levi, Golden, Zerubavel and Fisher, 1966; Schabel, Skipper, Laster and Thompson, 1966; Miller, Molovanu, Kaplan and Tocci, 1968; Hill and Littlejohn, 1970; Mempel and Thierfelder, 1970; Siegel and Morton, 1970), despite the immunosuppressive nature of such sera (Witz, Yagi and Pressman, 1968). We recently found that passive therapy with antileukaemia sera can give positive results only when the highest possible number of cytolytic potency units is administered (Reif and Kim, 1969). To prepare highly potent antisera, both immunization schedules and the dependence of the specificity of antisera on the types of leukaemias injected (Mohos and Kidd, 1957) require investigation. In a separate investigation (Kim and Reif, 1971), we have studied immunization schedules. In the present paper, the requirements for specificity of antisera, in terms of the strain of

origin of the injected leukaemia cells, are examined. This provides further insight into the preparation of such antisera.

We previously determined the specificity of rabbit antisera to thymic, splenic and leukaemic lymphocytes (Asakuma and Reif, 1968). Cross-reactions between antisera prepared against leukaemia cells and normal lymphoid cells, rather than cross-reactions between antisera prepared against leukaemia cells from different inbred strains, were studied. We found that unabsorbed antisera to leukaemias showed little or no specificity against leukaemia cells relative to non-thymic lymphocytes, but specificity appeared after absorption with a mixture of liver, kidney, and spleen. Antisera prepared against normal tissues were also tested (Asakuma and Reif, 1968).

In this study, antisera have been prepared against four different leukaemias, against a mixture of these leukaemias, and against an ovarian teratoma. These antisera were tested against each of the various leukaemias and against certain normal tissues. Two assays of antibody activity were used: immune cytolysis, and complement fixation.

MATERIALS AND METHODS

Mice and leukaemias

All mice were obtained from the Jackson Laboratory. Tumours and leukaemias were transplanted with sterile precautions in congenic strains. The route of transplantation was intraperitoneal (IP), unless stated otherwise.

Leukaemia C is an abbreviation for myeloid leukaemia C1498 II of C57BL/6 mice, obtained from Miss Brown of the Children's Cancer Research Foundation, Boston, and converted into the ascites form in our laboratory. Leukaemia M is an abbreviation for plasma cell neoplasm MOPC-21A of BALB/c mice, obtained in generation 89 from Dr Michael Potter of the National Cancer Institute, and transplanted subcutaneously. The C3HeB/Fe lymphosarcoma Gardner originated in a C3H mouse injected with estradiol benzoate (Gardner, Dougherty and Williams, 1944), and was transplanted in the low mammary tumour substrain C3HeB/Fe. E6A is an abbreviation for the ovarian teratoma E6496 of C3HeB/Fe mice, obtained in ascites form from Dr A. B. Griffen of the Jackson Laboratory. Lymphocytic leukaemia L1210 of DBA/2 mice was derived by Law, Dunn, Boyle and Miller (1949). Both Gardner and L1210 leukaemias were provided by Mr I. Wodinsky of Arthur D. Little, Inc. Leukaemia RA9 originated spontaneously in an AKR mouse; cells from the enlarged spleen and thymus were initially transplanted in our laboratory in May 1968.

The H-2 genotype of the strains of origin of the various tumours and leukaemias are as follows: C57BL/6, H-2^b; BALB/c and DBA/2, H-2^d; AKR, C3H and C3HeB/Fe, H-2^k (Snell and Stimpfling, 1966).

Antisera

Rabbits were immunized with mouse tissues by two courses of injection. Each course consisted of one subcutaneous injection of 1–2 ml of tumour cells incorporated in Freund-McDermott complete adjuvant (Reif and Norris, 1960), followed by five to seven intravenous injections (spaced over 3 weeks) of 5 ml of 1 per cent suspensions of viable leukaemia cells in isotonic saline. Antisera were inactivated by heating at 56° for 30 minutes and absorbed with 1/13 volume of AKR plasma, followed by three absorptions, each with 1/10 volume of packed mouse erythrocytes for 30 minutes at room temperature, as previously

described (Reif and Allen, 1964). Normal rabbit serum (NRS) was inactivated but not absorbed. Antisera were stored in small portions at −20° without preservative.

Rabbit antisera were tested either whole (-w in Tables 1 and 2), or else γG fractions (-γG) were prepared by precipitation with 40 per cent ammonium sulphate, followed by batch-fractionation with DEAE-cellulose (Reif, 1969). Antisera to leukaemia M and ovarian tumour E6A were treated similarly, except that ammonium sulphate precipitation was omitted. Antiserum X was prepared by injection of a mixture of leukaemias RA9, Gardner, L1210, C and M.

Cytolysis assay system

A small-scale assay system (Reif and Allen, 1964) was used. In outline, each assay tube contained 100,000 viable lymphocytes, suitably absorbed 10 per cent complement (Reif, 1962), and various dilutions of antiserum. Tubes were incubated for 1 hour at 37° and placed in ice water. For each tube in turn, the supernatant was sucked off the cells that had settled, 0·02 ml of vital dye was added, and 200 cells were classified under the microscope as stained or unstained. Results, in percentage of stained cells, were plotted against final antiserum concentration (per cent), and the cytolytic titre was read at 50 per cent stained cells, with a correction for stained cells in the control tube (Reif, 1962). The potency* of an antiserum was expressed as 100 divided by the cytolytic titre obtained in this assay system (Reif and Allen, 1964).

Complement fixation

A small-scale modification (Asakuma and Reif, 1968) of the complement (C) fixation method of Mayer (1961) was used.

In outline, serial doubling dilutions of antiserum were assayed for complement fixation with known numbers of tissue cells, or else with a known wet weight of a tissue homogenate. Disappearance of complement was determined by the failure of antibody-sensitized sheep erythrocytes, which were added later, to haemolyse. Reaction conditions and controls were as previously described (Asakuma and Reif, 1968).

The final concentration of antiserum in the experimental tubes was plotted against per cent haemolysis on semilogarithmic paper. From this plot, the C fixation titre of the antiserum was read as the final percentage concentration of antiserum at 50 per cent haemolysis, after correction for C fixation in control tubes. The C fixation potency* was calculated as 100 divided by the C fixation titre (Asakuma and Reif, 1968).

RESULTS

IMMUNE CYTOLYSIS

Rabbit antisera were prepared by hyperimmunization of groups of rabbits with different leukaemias or tumours. Both whole antisera and their γG fractions (named respectively -w and -γG in Table 1) were tested against various leukaemias, an ovarian tumour, and normal lymph node cells (Table 1).

* The reason for a conversion from 'titre' to 'potency' is that titres are difficult to visualize, since the stronger an antiserum the lower its titre. Use of the reciprocal of titre which we have called potency (Reif and Allen, 1964), is more meaningful, because the potency increases in direct proportion to the strength of the antiserum. A factor of 100 is used to make the 'potency' numerically equal to the final dilution of the antiserum.

TABLE 1

POTENCIES OF RABBIT ANTISERA PREPARED AGAINST LEUKAEMIAS OF SEVERAL MOUSE STRAINS, AS TESTED BY IMMUNE CYTOLYSIS AGAINST LEUKAEMIAS AND NORMAL LYMPHOCYTES FROM THESE STRAINS

Column No.:		1	2	3	4	5	6	7	8
		Potencies of antisera for immune cytolysis of the following cell types							
Rabbit serum*	After immunization against	C57BL/6 leukaemia C	C3HeB/Fe leukaemia Gardner	DBA/2 leukaemia L1210	C3HeB/Fe ovarian ascites tumour E6A	ARK leukaemia RA9	C57BL/6 lymph node cells	C3HeB/Fe lymph node cells	DBA/2 lymph node cells
NRS-w		6·3	< 3·3	< 3·3	7·4	< 3·3			< 3·3
NRS-γG		< 3·3	< 3·3	< 3·3	< 3·3	< 3·3			< 3·3
C-γG	C57BL/6 leukaemia C	208	192	88	48	170	160	124	118
TM-γG	BALB/c leukaemia M		800	491	371	860	460	832	692
G-γG	C3HeB/Fe leukaemia Gardner	371	741	70	39	588	126	285	92
E6A-γG	C3HeB/Fe ovarian tumour E6A	473	711	311	694	610	367	436	202
L-γG	DBA/2 leukaemia L1210	530	541	694	333	735	404	646	804
X-w	Mixed leukaemias	2056	1613	1133	410	1515	883	1537	2466
X-γG	Mixed leukaemias	1002	843	655	221	786	377	723	760

* Either whole serum (-w) or the γG fraction (-γG) was tested, as described under Methods. All results represent the mean of two experiments.

TABLE 2

POTENCIES OF RABBIT ANTISERA PREPARED AGAINST LEUKAEMIAS OF SEVERAL MOUSE STRAINS, AS TESTED BY C FIXATION AGAINST LEUKAEMIAS AND NORMAL LYMPHOCYTES AND TISSUE HOMOGENATES FROM THESE STRAINS

Column No.:		1	2	3	4	5	6	7	8	9	10	11
		Potencies of antisera for 50 per cent C fixation by the following cell types										
Rabbit serum*	After immunization against	C57BL/6 leukaemia C	C3HeB/Fe leukaemia Gardner	DBA/2 leukaemia L1210	DBA/2 lymph node	DBA/2 RBC	DBA/2 liver	DBA/2 kidney	DBA/2 brain	DBA/2 testes	DBA/2 skeletal muscle	DBA/2 lung
NRS-w			<3·3	71	68	<3·3	41	49	<3·3	34	<3·3	29
NRS-γG		<3·3	<3·3	103	9	<3·3	<6	<3·3	<3·3	<3·3	<3·3	<3·3
C-γG	C57BL/6 leukaemia C	131	117	680	371	<6	268	259	175	280	114	283
TM-γG	BALB/c leukaemia M	416	396	1785	2084	<6	1054	1355	597	1271	208	1430
G-γG	C3HeB/Fe leukaemia Gardner	244	421	1023	534	<6	489	451	218	297	114	348
E6A-γG	C3HeB/Fe ovarian tumour E6A	257	289	1396	1541	<6	60	622	427	2581	<6	633
L-w	DBA/2 leukaemia L1210	915	357	8600	1887	174	855	1080	1671	1086	374	963
L-γG	DBA/2 leukaemia L1210	254	216	3624	603	<6	281	650	220	465	15	520
X-w	Mixed leukaemias	1064	841	7940	3217	282	1163	1426	1653	1712	498	1204
X-γG	Mixed leukemias	513	405	2302	1244	<6	299	471	250	687	61	488

* See footnote of Table 1.

Normal rabbit serum or its γG fraction (top two lines, Table 1) was either inactive, or else active at a very low potency, when tested similarly (Table 1).

Antiserum C-γG (third line) was the weakest antiserum tested. While it reacted most strongly with the homologous leukaemia C, the reaction was almost as strong against leukaemias Gardner and RA9, which are of different H-2 genotype (both k rather than b). The reaction against leukaemia L1210 of DBA/2 mice was weak. Similarly low potencies against leukaemia L1210 were also obtained with other antisera (see below); possible reasons for this are given in the Discussion. The reaction of antiserum C-γG with normal lymph node lymphocytes (columns 6, 7 and 8) was strongest with the homologous (C57-BL/6) cells.

Antiserum TM-γG (fourth line) reacted more strongly against the H-2^k leukaemias Gardner and RA9 than to leukaemia L1210, which originated in the same strain (H-2^d) as the leukaemia against which the antiserum was prepared.

Antiserum G-γG (fifth line) reacted almost as strongly against another H-2^k leukaemia, RA9, as against the homologous leukaemia cells. The reaction against normal lymphocytes was strongest against those of homologous genotype (column 7). There was little cross-reaction with an ovarian teratoma which originated in the same strain. Again, the reaction against leukaemia L1210 (which differs in H-2 genotype) was remarkably low.

Antiserum E6A γG (sixth line) reacted similarly, except that the reaction against the homologous ovarian tumour cells was far stronger. The higher potency seen versus leukaemia L1210 may well reflect similarly higher potencies seen in the reactions with normal lymphocytes (columns 6, 7 and 8).

Antiserum L-γG (seventh line), on the basis of a stronger reaction against homologous (DBA/2) normal lymph node cells (than against those of other strains: columns 6, 7 and 8), was expected to react most strongly against the homologous (L1210) leukaemia cells. This was not the case (columns 1, 2, 3 and 5). This result is in line with the lower reactivities against L1210 cells also found for antisera prepared against other leukaemias (see above).

Antiserum X (last two lines, Table 1), from rabbits immunized with several leukaemias, showed as strong or stronger cytolytic potencies against the various leukaemias, than did any other antiserum tested.

C FIXATION

We previously used C fixation to evaluate the specificity of antilymphocyte sera (Asakuma and Reif, 1968). While immune cytolysis proved superior for this purpose, C fixation gives additional information and permits estimation of antibody binding with non-viable tissue residues, which is not possible with immune cytolysis. The same antisera were therefore tested by C fixation against leukaemias of three different H-2 genotypes, and against various normal DBA/2 tissues (Table 2).

Normal rabbit serum (first two lines, Table 2) showed weak reactivity against leukaemia L1210.

Antiserum C-γG (third line) showed stronger C fixation with leukaemia L1210 than with other leukaemias. Similar results were obtained with other antisera (see below). Such results are in direct contrast to those obtained by immune cytolysis (Table 1); an explanation is suggested in the Discussion.

Antiserum TM-γG (fourth line) reacted strongly against leukaemia L1210. Reactions against normal tissue residues were of comparable potencies to the reaction against leukaemias.

Antiserum G-γG (fifth line) reacted more strongly against the homologous leukaemia than against leukaemia C. However, the reactions of this serum, and of *Antiserum E6A-γG* (sixth line), were strongest against leukaemia L1210.

Antiserum L (seventh line) reacted very strongly with the homologous leukaemia L1210. Also, *Antiserum X* (last two lines, Table 2) prepared against mixed leukaemias, reacted very strongly with all leukaemias, but especially strongly with leukaemia L1210. The reactions of all antisera with normal tissue residues was relatively strong, and decreased in approximately the following order: testis, kidney, liver, lung, brain, muscle, with very little reaction against erythrocytes.

EXHAUSTIVE ABSORPTION OF ANTI-L1210 SERUM

A potent rabbit antiserum prepared against L1210 leukaemia cells was absorbed with 1/4 volume of packed mouse erythrocytes. Portions of this absorbed antiserum (AA) were further absorbed with lymphocytes (lymph node, splenic and thymic) and once again with washed residues of liver and kidney. The ratio of the number of DBA/2 mice from which these tissues were derived to the volume of antiserum (ml) varied as follows: (a) 1:10, (b) 1:3, (c) 1:1, (d) 3:1. All absorptions were done for 30 minutes at 37°. Each portion of separately absorbed antiserum was tested by immune cytolysis against L1210 cells, lymph node lymphocytes, and splenic lymphocytes.

Relative to antiserum AA, the mean cytolytic potencies of portions absorbed according to schedules (a), (b), (c) and (d) above were 47, 25, 12 and 5·6 per cent, respectively, when determined against L1210 cells. For any portion of antiserum, the cytolytic potency against L1210 cells, divided by the cytolytic potency against lymph node lymphocytes, was a measure of the specificity of a particular portion of antiserum against leukaemia cells relative to normal lymphocytes (Reif, Allen and McVety, 1965). The values of this 'specificity ratio' for antiserum portions (a), (b), (c) and (d) were 0·54, 0·72, 0·74 and 1·26, compared to 0·65 for antiserum AA. The equivalent specificity ratios for the reaction against L1210 cells relative to splenic lymphocytes were 1·29, 1·38, 1·57 and 2·34, compared to 0·90 for antiserum AA. When compared to the reaction with either lymph node or splenic lymphocytes, a substantial increase in specificity against L1210 leukaemia cells was thus obtained only with schedule (d), which represents very thorough absorption and concomitantly high loss of cytolytic potency.

DISCUSSION

Heterologous antisera have long been used for the treatment of human leukaemia (Lindstrom, 1927; Brittingham and Chaplin, 1960). While interest has quickened recently (Sekla, Holecková, Janele, Libnásky and Hnevkovsky, 1967; Tsirimbas, Pfisterer, Hornung, Thierfelder, Michlmayr and Stich, 1969; Fernbach, Rossen and Butler, 1970; Mempel and Thierfelder, 1970), there is no convincing evidence that such sera produce or prolong remissions in human leukaemia. The present model study in mice is not concerned with the *in vivo* effectiveness of such sera, but helps to answer questions that arise in their preparation. For instance, must antisera to human leukaemias be prepared by immunizations of animals with leukaemias of the same HL-A type as the patient to be treated? Can immunization with a mixture of leukaemias produce antisera potent against leukaemias of different HL-A types? Resolution of these questions must await experimentation in man. However, there is a sufficiently close parallel between the H-2 system

of mice and the HL-A system of man, that work with mice is apt to provide useful suggestions as to the likely outcome of equivalent work in man.

CELLULAR ANTIGENS

What murine antigens are able to stimulate formation of antibodies in rabbits? Four classes of cell surface antigens come into consideration: allogenic, organ-specific, tumour-specific and species-specific.

With few exceptions (Snell and Stimpfling, 1966), allogenic specificities such as H-1 to H-11, TL and θ are expressed on the cell surface of the corresponding leukaemias. To predict the cross-reactions of antisera prepared against different leukaemias, it must be remembered that mouse strains (and therefore also leukaemias) of different H-2 genotype often share some H-2 antigens. For instance, leukaemia C (H-2^b) shares H-2 specificity No. 5 with leukaemias Gardner and RA9 (both H-2^k) and specificities Nos. 6, 14, 27, 28 and 29 with leukaemia L1210 (H-2^d), while L1210 shares specificities Nos. 3 and 8 with leukaemias Gardner and RA9 (Snell and Stimpfling, 1966).

In the present study, antisera prepared by immunization with a leukaemia of a certain genotype were most potent for immune cytolysis of leukaemias of the same H-2 genotype in the case of antisera G and E6A (Table 1). Similarly, antiserum TM reacted more strongly against L1210 cells (also of genotype H-2^d) than all sera other than those prepared by injection of L1210 cells (sera L and X). The results for antisera C and L are not readily evaluated, since these were the only leukaemias tested that were of H-2^b and H-2^d genotypes, respectively.

With regard to the two allogenic specificities θ-AKR find θ-C3H, we were only able to differentiate these with heterologous antisera after exhaustive absorptions (Asakama and Reif, 1968). It seems questionable whether other alloantigens, such as the PC alloantigens of plasma cells (Takahashi, Old and Boyse, 1970), can be detected with heterologous antisera without similarly extensive absorptions. While such alloantigens possess characteristic organ distributions, the term 'organ-specific' should probably be reserved for antigens present only in a single organ, or else in cells of closely related type.

Tumour-specific transplantation antigens (Gorer, 1938; Boyse, Old, Stockert and Shigeno, 1968; Klein, 1969; Watson, Ralph, Sarkar and Cohn, 1970) are relatively weakly immunogenic on allogenic immunization. Nevertheless, the more difficult feat of demonstrating such antigens with heterologous antisera has been accomplished for chronic lymphocytic leukaemia (Bentwitch, Cohen, Sulitzeanu, Izak and Weiss, 1970). No evidence of specificity to such antigens, nor to the species-specific antigens T, C and F (Boyle and Davies, 1966) and MSLA (Shigeno, Hämmerling, Arpels, Boyse and Old, 1968) was obtained here.

In the present study, an antiserum with high cytolytic potency against leukaemias of three different H-2 genotypes was prepared by immunization with a mixture of leukaemia cells. Previously, a close correlation between the results of *in vitro* immune cytolysis and *in vivo* antileukaemia therapy has been found (Mohos and Kidd, 1957; Reif and Kim, 1969). Therefore, such an antiserum would be expected to be effective *in vivo*. Finally, the present data suggest that heteroimmunization with a mixture of leukaemia cells from different patients might well result in antisera with high *in vitro* potencies against human leukaemias of different HL-A types.

RELATIONSHIP BETWEEN C FIXATION AND IMMUNE CYTOLYSIS

An interesting facet of the data is the apparent contradiction between the consistently low potencies of various antisera for immune cytolysis of L1210 cells (Table 1) and the consistently high potencies for C fixation by the same cells (Table 2). The discrepancies in this connection were smaller for the other leukaemias. How can these data be explained?

Recent work on immune lysis of erythrocytes or leukaemia cells suggests that after attachment to antigen sites on the cell surface, two molecules of the usual type of γG (Borsos and Rapp, 1965a), a single molecule of γM (Borsos and Rapp, 1965b), or a single molecule of an unusual type of γG (Frank and Gaither, 1970) are able to fix the first component of complement, C_1. To explain why the efficiency of C_1 for haemolysis of γM-sensitized sheep erythrocytes decreases dramatically as the density of antigen sites decreases (Linscott, 1970), it is suggested that *two* sites of antibody attachment to the cell

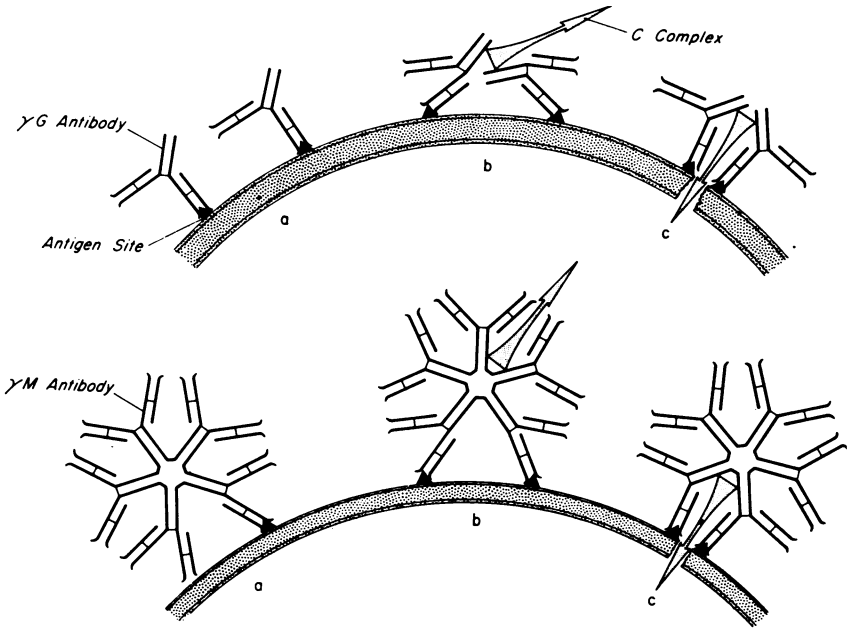

FIG. 1. A molecular model of immune cytolysis. It is suggested that penetration of the cell surface can occur only if the complement (C) complex is bound by one (γM) or two (γG) antibody molecules attached to closely-spaced antigen sites, and if the C complex is oriented to make optimal contact with the cell surface. Antibody binding is illustrated (a) without binding of C, (b) with lytically ineffective binding of C, and (c) with lytically effective binding of C.

surface are necessary before C_1 can be fixed by any type of antibody. In the case of γM, a single molecule would attach to two adjacent antigen sites (Fig. 1), while the unusual property of 'unusual' γG (Frank and Gaither, 1970) might be a flexible structure that permits two-point contact with the cell surface.

The above work suggests that lytically effective binding of the C complex can occur only when two antigen sites are relatively closely spaced. However, fixation of C_1 to cell-bound molecules of antibodies does not guarantee either binding of the remaining C components or sufficient damage of the membrane to lyse the cell (Linscott, 1970). The effi-

ciency of this latter step is 30–50 per cent with γG-sensitized sheep erythrocytes (Rapp and Borsos, 1966), but only about 0·03 per cent with γM-sensitized mouse leukaemia cells (Borsos, Colten, Spalter, Rogentine, and Rapp, 1968), a 1000-fold difference. To explain these data as well as our own, it is suggested that immune lysis can occur only when the antibody-bound complement complex is correctly oriented for optimal (and presumably close) contact with the cell surface. The illustration of this (Fig. 1) has been arranged to fit current concepts on the structure of antibody molecules (Svehag, Chesebro and Bloth, 1967; Green, 1969) and the attachment of C_1 to the Fc portion of γG or γM antibodies (Weiser, Myrvik and Pearsall, 1969).

According to the above explanation, the present anomalous data with L1210 would be explicable on the basis of a lower surface antigen concentration on L1210 cells relative to other mouse leukaemias: sufficient L1210 antigen sites would be available to fix 50 per cent of the small quantity of C added in the C fixation test (especially since 10 times more cells were used than in the cytolysis assay), but would be insufficient to provide as ready immune cytolysis, with its more stringent requirement for closely-spaced antigen sites. Since different cell types possess widely different sensitivites to non-immune lysis, implying differences in the strength of different cell surfaces (Reif and Allen, 1966), the sensitivities to immune lysis may also depend on this physical characteristic.

ACKNOWLEDGMENT

This work was supported by Public Health Service Research Grant No. CA-04469 AI from the National Cancer Institute.

REFERENCES

Asakuma, R. and Reif, A. E. (1968). 'The specificity of antilymphocyte sera to thymic, splenic, and leukemic lymphocytes.' *Cancer Res.*, **28**, 707.

Bentwitch, Z., Cohen, I., Sulitzeanu, D., Izak, G. and Weiss, D. W. (1970). 'Antigenicity of lymphocytes from chronic lymphocytic patients and from normal newborns.' *Proc. Amer. Ass. Cancer Res.*, **11**, 7.

Borsos, T., Colten, H. R., Spalter, J. S., Rogentine, N. and Rapp, H. J. (1968). 'The C'1a fixation and transfer test: examples of its applicability to the detection and enumeration of antigens and antibodies at cell surfaces.' *J. Immunol.*, **101**, 392.

Borsos, T. and Rapp, H. J. (1965a). 'Complement fixation on cell surfaces by 19S and 7S antibodies.' *Science*, **150**, 505.

Borsos, T. and Rapp, H. J. (1965b). 'Hemolysin titration based on fixation of the activated first component of complement: evidence that one molecule of hemolysin suffices to sensitize an erythrocyte.' *J. Immunol.*, **95**, 559.

Boyle, W. and Davies, D. A. L. (1966). 'Antigens of the surface of mouse ascites tumour cells. I. Studies with rabbit anti-mouse cell sera.' *Immunology*, **11**, 353.

Boyse, E. A., Old, L. J., Stockert, E. and Shigeno, N. (1968). 'Genetic origin of tumor antigens.' *Cancer Res.*, **28**, 1280.

Brittingham, T. E. and Chaplin, H., Jr (1960). 'Production of a human "antileukemic leukocyte" serum and its therapeutic trial.' *Cancer*, **13**, 412.

Fernbach, D. J., Rossen, R. D. and Butler, W. T. (1970). 'Studies of antilymphocyte globulin (ALG) treatment of children with acute leukemia (AL).' *Proc. Amer. Ass. Cancer Res.*, **11**, 25.

Frank, M. M. and Gaither, T. A. (1970). 'Complement fixation by a single molecule of γG hemolysin.' *J. Immunol.*, **104**, 1458.

Gardner, W. U., Dougherty, T. F. and Williams, W. L. (1944). 'Lymphoid tumors in mice receiving steroid hormones.' *Cancer Res.*, **4**, 73.

Gorer, P. A. (1938). 'The antigenic basis of tumor transplantation.' *J. Path. Bact.*, **47**, 231.

Green, N. M. (1969). *Advances in Immunology* (Ed. by F. J. Dixon, Jr and H. G. Kunkel), p. 1. Academic Press, New York.

Hill, G. J., II and Littlejohn, K. (1970). 'Comparison of heterologous antiserum and cyclophosphamide in therapy of mouse leukemia.' *Proc. Amer. Ass. Cancer Res.*, **11**, 37.

Kim, C.-A. H. and Reif, A. E. (1971). 'Immunization schedules for potent rabbit antisera to leukemia L1210.' *Cancer Res.*, **31**, 7.

Klein, G. (1969). 'Experimental studies in tumor immunology.' *Fed. Proc.*, **28**, 1739.

Law, L. W., Dunn, T. B., Boyle, P. J. and Miller, J. H. (1949). 'Observations on the effect of a folic acid antagonist on transplantable lymphoid leukemias in mice.' *N. nat. Cancer Inst.*, **10**, 179.

Levi, E., Golden, R., Zerubavel, R. and Fischer, J. (1966). 'The activity of tolerant antileukemia sera. I. *in vitro* effects.' *Cancer Res.*, **26**, 659.

Lindstrom, G. A. (1927). 'An experimental study of myelotoxic sera. Therapeutic attempts in myeloid leukemia.' *Acta med. scand.* (suppl.), **22**, 1.

LINSCOTT, W. D. (1970). 'An antigen density effect on the hemolytic efficiency of complement.' *J. Immunol.*, **104**, 1307.

MAYER, M. M. (1961). 'Complement and complement fixation.' *Experimental Immunochemistry* (Ed. by E. A. Kabat), 2nd edn, p. 133. Thomas, Springfield.

MEMPEL, W. and THIERFELDER, S. (1970). 'Die Wirkung von Antilymphozytenserum allein oder in Verbindung mit einem Zytostatikum auf eine Mauseleukaemie.' *Blut*, **20**, 37.

MILLER, D. G., MOLOVANU, G., KAPLAN, A. and TOCCI, S. (1968). 'Antilymphocytic leukemic serum and chemotherapy in the treatment of murine leukemia.' *Cancer*, **22**, 1192.

MOHOS, S. C. and KIDD, J. G. (1957). 'Effects of various immune rabbit serums on the cells of several transplanted mouse lymphomas in vitro and in vivo.' *J. exp. Med.*, **105**, 233.

RAPP, H. J. and BORSOS, T. (1966). 'Forssman antigen and antibody: preparation of water soluble antigen and measurement of antibody concentration by precipitin analysis, by C'1a fixation and by hemolytic activity.' *J. Immunol.*, **96**, 913.

REIF, A. E. (1962). 'Immune cytolysis of three mouse ascites tumors.' *J. Immunol.*, **89**, 849.

REIF, A. E. (1969). 'Batch preparation of rabbit γG globulin with DEAE-Cellulose.' *Immunochemistry*, **6**, 723.

REIF, A. E. and ALLEN, J. M. (1964). 'The AKR thymic antigen and its distribution in leukemias and nervous tissues.' *J. exp. Med.*, **120**, 413.

REIF, A. E. and ALLEN, J. M. (1966). 'The fragility of normal and leukemic lymphocytes of AKR mice: a study of cell injury by physical agents.' *Cancer Res.*, **26**, 131.

REIF, A. E., ALLEN, J. M. V. and MCVETY, L. M. (1965). 'A general method for the calculation of serological specificity.' *Immunology*, **8**, 384.

REIF, A. F. and KIM, C.-A. H. (1969). 'Therapy of transplantable mouse leukaemias with antileukaemia sera.' *Nature (Lond.)*, **223**, 1377.

REIF, A. E. and NORRIS, H. J. (1960), 'A system for quantitative determination of cytotoxic activity of antisera to ascites tumor cells.' *Cancer Res.*, **20**, 1235.

SCHABEL, F. M., JR, SKIPPER, H. E., LASTER, W. R., JR and THOMPSON, S. A. (1966). 'Experimental evaluation of potential anticancer agents. XX. Development of immunity to leukemia L1210 in BDF1 mice and effects of therapy.' *Cancer Chem. Rep.*, **50**, 55.

SEKLA, B., HOLECKOVÁ, E., JANELE, J., LIBÁNSKÝ, J. and HNEVKOVSKÝ, O. (1967). 'The effects of heterologous immune globulins upon human cancer in vivo.' *Neoplasma*, **14**, 641.

SHIGENO, N., HÄMMERLING, U., ARPELS, C., BOYSE, E. A. and OLD, L. J. (1968). 'Preparation of lymphocyte-specific antibody from anti-lymphocyte serum.' *Lancet*, **ii**, 320.

SIEGEL, B. V. and MORTON, J. I. (1970). 'Rauscher viral leukemogenesis in BALB/c mice treated with rabbit antimouse thymocyte serum.' *J. nat. Cancer Inst.*, **44**, 573.

SNELL, G. D. and STIMPFLING, J. H. (1966). 'Genetics of tissue transplantation.' *Biology of the Laboratory Mouse* (Ed. by E. L. Green), 2nd edn, p. 457. McGraw-Hill, New York.

SVEHAG, S.-E., CHESEBRO, B. and BLOTH, B. (1967). 'Ultrastructure of gamma M immunoglobulin and alpha macroglobulin: electron-microscopic study.' *Science*, **158**, 933.

TAKAHASHI, T., OLD, L. J. and BOYSE, E. A. (1970). 'Surface allo-antigens of plasma cells.' *J. exp. Med.*, **131**, 1325.

TSIRIMBAS, A. D., PFISTERER, H., HORNUNG, B., THIERFELDER, S., MICHLMAYR, G. and STICH, W. (1969). 'Studies with heterologous antilymphocytic serum as a therapy for chronic lymphocytic leukemia.' *Blut*, **19**, 420.

WATSON, J., RALPH, P., SARKAR, S. and COHN, M. (1970). 'Leukemia viruses associated with mouse myeloma cells.' *Proc. nat. Acad. Sci. (Wash.)*, **66**, 344.

WEISER, R. S., MYRVIK, Q. N. and PEARSALL, N. N. (1969). *Fundamentals of Immunology*, p. 25. Lea & Febiger, Philadelphia, Pa.

WITZ, I., YAGI, Y. and PRESSMAN, D. (1968). 'Immunosuppressive activity of rabbit antisera directed against mouse lymphocytic leukemia L1210.' *Proc. Soc. exp. Biol. (N.Y.)*, **127**, 562.