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Cytolysis of Mouse Lymph Node Cells by Alloantibody: A Comparison of Guinea-Pig and Rabbit Complements

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Summary. Rabbit serum obtained from selected animals, and absorbed with mouse liver and spleen, was used as a source of complement in the ^{51}Cr monitored cytolysis of mouse lymph node cells by H-2 alloantibody. Antibody was more efficiently cytolytic in the presence of rabbit complement (RC′) than in the presence of guinea-pig complement (GPC′). Addition of rabbit or goat anti-mouse globulin to GPC′ caused a modest increase in the cytolytic efficiency of the alloantibody. Addition of antiglobulin to RC′ reduced the cytolytic efficiency of alloantibody. The slope of the dilution curve of H-2 antibody was less steep in the presence of RC′ than in the presence of GPC′. The apparent order of reaction with respect to antibody was lower with RC′ than with GPC′. Mouse heteroantibody against sheep erythrocytes was slightly more haemolytically efficient in the presence of GPC′ than in the presence of RC′. The dilution curves of heteroantibody in the presence of RC′ or GPC′ had the same slope, and the apparent orders of reaction with respect to antibody were similar. A greater concentration of GPC was required to complement the alloantibody than the heteroantibody whereas a similar concentration of RC′ sufficed in the two systems. Possible interpretations of these results are discussed.

INTRODUCTION

Mouse isoantibody directed against antigens of the H-2 histocompatibility locus has since 1956 been measured by its ability to kill lymphoid cells in the presence of guinea-pig complement (Gorer and O'Gorman, 1956). The technique as originally described, detected cell death by loss of the ability to exclude Trypan Blue. In 1965 Wigzell introduced the use of ^{51}Cr (sodium chromate) for labelling of target cells. Cell death is detected in this technique by the liberation from the cell of incorporated radioactivity. Sanderson (1965) made a detailed study of the kinetics of lymphoid cell lysis using ^{51}Cr and concluded that the reaction proceeded in an identical fashion with haemolysis of sheep red blood cells by heterologous antibody. He concluded that the kinetics of complement action described in the latter system were applicable also to the former. Chromium monitored cytolysis has since been widely used for the measurement of histocompatibility antibody and for following purification of histocompatibility antigens (Nathenson and Davies, 1966; Kandutsch, Hilbert, Ruskiewicz, Cherry and Snell, 1968; Nathenson, 1968; Davies, 1968). The advantages of the technique are its high precision and reproducibility. Its

major disadvantage is its relatively low sensitivity. It is applicable to measurement of antibodies of the H-2 system and of some non-H-2 systems (Lilly and Nathenson, 1968; Rogentine and Plocinik, 1967) and to the tumour-specific antigen of Moloney virus-induced lymphoma cells (Haughton, 1965). We have been concerned with an attempt to increase the sensitivity of chromium monitored cytolysis without sacrificing its innate precision. Following our own preliminary observation and isolated reports in the literature (Hildemann, 1957; Boyse, Old and Stockert, 1962; Winn, 1965), we have studied the system using rabbit complement in place of the usual guinea-pig complement. This has resulted in a marked increase in the cytolytic efficiency of mouse isoantibody. We have studied the kinetics of the reaction and whilst our data do not permit final conclusions as to the mode of action of rabbit complement, we are able to exclude some of the possibilities. The use of rabbit complement enables us to work with weak antisera in which antibody could not be measured by the conventional system and to use smaller amounts of stronger antisera. The results of our studies are presented in this report.

MATERIALS AND METHODS

Mice

Inbred mice were obtained from Dr Bernard Amos's colony at Duke University.

Antisera

CBA-EL4 antiserum was prepared by injecting CBA mice intraperitoneally (i.p.) at weekly intervals for over a month with suspensions of EL4, a long transplanted C57 ascites lymphoma. One week after the final injection, the mice were bled from the retro-orbital sinus with a Pasteur pipette. The serum was collected, pooled, and stored at $-20°$.

C57-BP8 antiserum was prepared by injecting C57BL/10 mice i.p. at weekly intervals for over a month with suspensions of BP8, a C3H sarcoma. The mice were bled 1 week after the final injection and the serum collected and stored in the usual manner.

CBA-SRBC antiserum was prepared by injecting CBA females with 0·2 ml of 30 per cent (v/v) washed sheep red blood cells i.p. at weekly intervals for at least 4 weeks. The mice were bled 1 week after the final injection and the serum collected and stored in the usual manner.

C57-HTH antiserum was prepared by injecting C57BL/10 females and males i.p. with 2/5 mouse equivalents of an HTH spleen, lymph node and thymus cell suspension. This was followed by an i.p. injection of 3/5 mouse equivalents of the same type of cell suspension on the 3rd day. The mice were bled on the 7th day and the serum collected.

Rabbit-mouse globulin was prepared by injecting New Zealand White rabbits with mouse globulin which had been precipitated with 50 per cent saturated ammonium sulphate solution. The first injection was given intravenously (i.v.). The second injection was given intramuscularly (i.m.) 11 days after the first injection, and the rabbits were bled 6 days later. The rabbits were then given two i.m. injections 1 week apart of mouse globulin emulsified in an equal volume of complete Freund's adjuvant. Eleven days after the final injection the rabbits were bled and the serum collected, pooled, and stored in the usual manner.

Destruction of 19S antibody by 2-mercaptoethanol

The method of Uhr and Finkelstein (1963) was used. Treated antisera were dialysed overnight against cold saline to remove excess 2-mercaptoethanol.

Preparation of cell suspensions

Lymph node cells and thymus cells. Lymph nodes or thymus were minced with scissors in tissue culture medium and gently sucked up and down in a syringe to release the cells. The cells were washed three times and adjusted to the appropriate concentration in tissue culture medium containing 5 per cent foetal calf serum.

Sheep red blood cells. Sheep red blood cells in (sterile) Alsever's solution were obtained from Robbin Laboratories, Incorporated, Chapel Hill, North Carolina. The cells were washed three times and adjusted to the appropriate concentration.

Tumour cells. Ascites tumour cells were obtained by washing out the peritoneum of tumour bearing mice with saline.

Radioactive isotope

^{51}Cr in the form of $Na_2{}^{51}CrO_4$ in isotonic saline was obtained from the Radiochemical Centre in Amersham, England (catalogue No. CJS-1P). The specific activity varied between 90 and 180 c/g of chromium. Each sample was diluted with isotonic saline to prepare a stock solution containing 2 mc/ml. The supernatants and residues from each titration were counted in a crystal scintillation counter (single channel, pulse height analyser—Tracerlab Gamma/guard, model GGA-100). The background was 10–20 counts/min.

Labelling cells

Lymph node cells. The lymph node cells were washed once, adjusted to 90×10^6 cells/ml, and labelled with 200 μc ^{51}Cr/ml. The cells were incubated at 37° for 1 hour, after which they were washed five times and adjusted to 10×10^6 cells/ml in tissue culture medium containing 5 per cent foetal calf serum for the titrations.

Red blood cells. The red blood cells were washed three times in saline. A volume of 0·6 ml of tissue culture medium was added to 0·2 ml of packed cells. The cells were labelled with 200 μc ^{51}Cr/ml at 37° for 1 hour and then washed six times in saline and diluted to 1 per cent for the test.

Complement source

Guinea-pig serum and rabbit serum were used as the sources of complement for the cytotoxicity titrations. In order to avoid obtaining rabbit serum toxic for mouse cells, the rabbits were very carefully screened. This was accomplished by testing sera from sample bleedings of rabbits against mouse lymph node cells in the ^{51}Cr cytotoxicity assay. Only those rabbits whose sera at a 1 : 16 dilution demonstrated less than 10 per cent ^{51}Cr release were selected as complement donors.

Complement absorption

Guinea-pig serum and rabbit serum were absorbed twice at 0° for 30 minutes with an insoluble fraction of mouse liver and spleen homogenate. (This insoluble fraction was obtained by mincing the mouse liver and spleen, mashing it through a stainless steel mesh, and washing the homogenate until the supernatant fluid was clear.) Ten volumes of serum were absorbed with 1 volume of the insoluble fraction, after which the serum was stored at −20°.

Cytotoxicity titration methods

Two-fold (2×), one and a half-fold (1·5×), and one and a quarter-fold (1·25×)

dilutions of antiserum were made with Lang–Levy micropipettes in 6×50 mm test tubes with tissue culture medium containing 5 per cent foetal calf serum (v/v).

One stage test

(1) One volume of the ^{51}Cr-labelled target cells was added to 1 volume of the serum dilution (1 volume = 30–45 μl).
(2) The tubes were shaken and incubated at 37° for 15 minutes in a water bath.
(3) One volume of absorbed guinea-pig or rabbit serum diluted in tissue culture medium was added as a source of complement, always in complement excess.
(4) The tubes were shaken and incubated at 37° for 30 minutes in a water bath.
(5) The tubes were centrifuged at 2000 ***g*** for 5 minutes.
(6) Two volumes of supernatant from each tube were transferred to a tube containing 0·5 ml of saline.
(7) The supernatants, residues, and background were counted in a crystal scintillation counter.

Two-stage test

(1) One volume of ^{51}Cr-labelled cells was added to 1 volume of serum.
(2) The tubes were shaken and incubated in a water bath at 37° for 30 minutes.
(3) The sensitized cells were diluted with tissue culture medium, centrifuged at 1000 ***g*** for 2 minutes and the supernatant removed.
(4) One volume of guinea-pig or rabbit serum diluted with tissue culture medium was added as a source of complement (in complement excess).
(5) The tubes were shaken and incubated at 37° for 45 minutes.
(6) The tubes were centrifuged at 2000 ***g*** for 5 minutes.
(7) One volume of supernatant from each tube was transferred to a tube containing 0·5 ml saline.
(8) The supernatants and residues were counted.

Calculation of percentage of ^{51}Cr released from the cells

$$\text{Per cent } ^{51}\text{Cr released} = \frac{\text{Supernatant count} - \text{Background} \times \text{correction factor}}{\text{Total count} - 2 \times \text{Background}}$$

$$\left(\text{Correction factor} = \frac{\text{Total supernatant}}{\text{Aliquot counted}}\right).$$

The percentage of ^{51}Cr released as calculated above may be plotted against the serum dilution to give a typical dilution curve.

RESULTS

ISOANTIBODY DILUTION CURVES USING LYMPH NODE CELLS AS TARGETS

Serial dilutions of antibody were incubated at 37° with equal volumes of ^{51}Cr-labelled lymph node cells (10^7/ml) for 15 minutes. An equal volume of 50 per cent absorbed guinea-pig serum or 25 per cent absorbed rabbit serum was then added as a supply of excess complement and incubation continued for a further 45 minutes. The tubes were then centrifuged at 2000 ***g*** for 5 minutes and a measured volume of supernatant removed for

counting in the scintillation counter. The radioactivity of the remaining supernatant plus sedimented cells and cell debris, was also counted to permit calculation of the percentage total ^{51}Cr liberated into the supernatant. For study of guinea-pig complement, serial dilutions of antiserum were prepared at 1·25-fold steps and for rabbit complement at 1·5-fold steps.

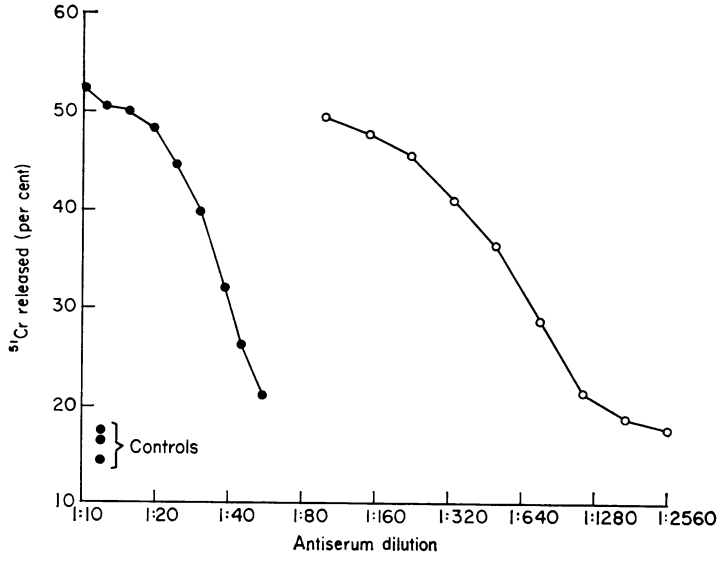

FIG. 1. Cytolysis of ^{51}Cr-labelled HTG (H-2^g) lymph node cells by serial dilutions of CBA (H-2^k) anti-EL4 (H-2^b lymphoma) serum in the presence of excess guinea-pig (●) or rabbit (○) complement. The points plotted are the arithmetic averages of sextuplicate determinations detailed in Tables 1 and 2.

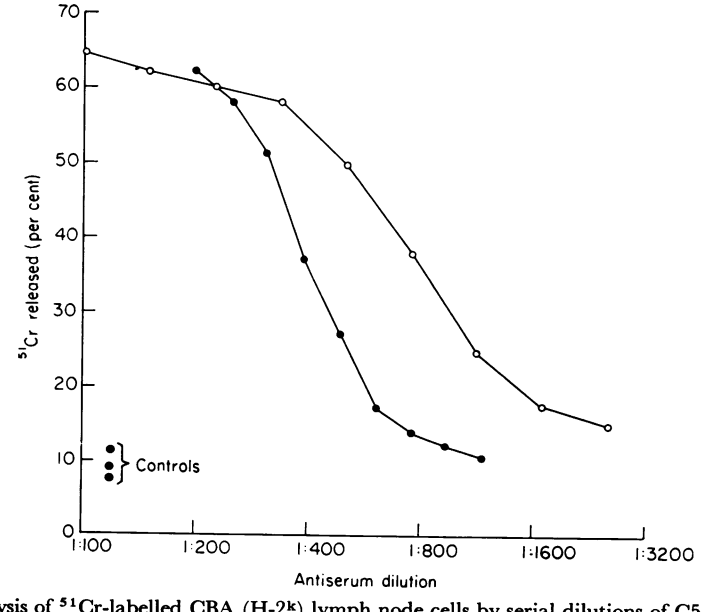

FIG. 2. Cytolysis of ^{51}Cr-labelled CBA (H-2^k) lymph node cells by serial dilutions of C57 (H-2^b) anti-BP8 (H-2^k sarcoma) serum in the presence of excess guinea-pig (●) or rabbit (○) complement. The points plotted are the arithmetic averages of sextuplicate determinations detailed in Tables 3 and 4.

TABLE 1

CYTOLYSIS OF HTG (H-2^g) LYMPH NODE CELLS BY 1·25-FOLD SERIAL DILUTIONS OF HYPERIMMUNE CBA (H-2^k) ANTI-EL4 (H-2^b LYMPHOMA)* SERUM IN THE PRESENCE OF EXCESS GUINEA-PIG COMPLEMENT

Experiment No.	Per cent of total ^{51}Cr liberated by serum diluted:									Without serum with C′	Without serum or C′
	1 : 10	1 : 12·5	1 : 15·6	1 : 19·5	1 : 24·4	1 : 30·5	1 : 38·1	1 : 47·7	1 : 57		
1	56	50·5	49·5	49·5	45	38	31	31	19·5	17	15
2	53	52·5	53	48	45	41	33·5	25	19	17	15
3	52·5	49	53	47·5	45·5	38·5	31	23	19·5	17·5	15·5
4	52	50·5	45	49	45·5	41	31·5	26	23·5	16	15
5	50·5	49	50·5	48	43	39·5	33	25	22	15·5	–
6	49	50	49	48	42	39·5	31·5	26·5	21	15·5	–
Arithmetic average	52·2	50·3	50	48·3	44·3	39·6	31·9	26·1	20·8	16·4	15·1

* Maximum reacting H-2 specificities: 2, 6, 14 and 22.

TABLE 2

CYTOLYSIS OF HTG (H-2^g) LYMPH NODE CELLS BY 1·5-FOLD SERIAL DILUTIONS OF HYPERIMMUNE CBA (H-2^k) ANTI-EL4 (H-2^b LYMPHOMA)* SERUM IN THE PRESENCE OF EXCESS RABBIT COMPLEMENT

Experiment No.	Per cent of total ^{51}Cr liberated by serum diluted:									Without serum with C′	Without serum or C′
	1 : 100	1 : 150	1 : 225	1 : 338	1 : 507	1 : 760	1 : 1140	1 : 1710	1 : 2565		
1	48	50	43	39	36	28	21	18	18	18	14·5
2	48·5	46	44	41	36	30	21	18	17	17·5	14·5
3	47	46	45·5	40	33	29	20	20	17	16	14
4	48	48	46	41	38	27	21	–	17	17·5	14
5	50	48·5	46	42	38	28	21	20	18	17·5	–
6	53	48·5	47·7	41	36	29	22·5	17·5	18	17·5	–
Arithmetic average	49·3	47·7	45·3	40·7	36·2	28·5	21·1	18·7	17·5	17·3	14·3

* Maximum reacting H-2 specificities: 2, 6, 14 and 22.

TABLE 3

CYTOLYSIS OF CBA (H-2^k) LYMPH NODE CELLS BY 1·25-FOLD SERIAL DILUTIONS OF HYPERIMMUNE C57 (H-2^b) ANTI-BP8 (H-2^k SARCOMA) SERUM IN THE PRESENCE OF EXCESS GUINEA-PIG COMPLEMENT

Experiment No.	Per cent of total ^{51}Cr liberated by serum diluted:									Without serum with C′	Without serum or C′
	1 : 200	1 : 250	1 : 312	1 : 390	1 : 488	1 : 610	1 : 762	1 : 952	1 : 1180		
1	61	58	54	41	28	20	14·5	12·5	11	8	9
2	63	60	52	32	28	16	14	11	10	9	9
3	60	59	52	38	27	16	17·5	15	12	8·5	8·5
4	–	57	50	38	23	15	8·5	13	11	8	9
5	60	57	50	38	27·5	18·5	14·5	12	10	9	
6	64	57	50	36	27·5	18·5	14	9·5	10	8·5	
Arithmetic average	62	58	51·3	37·1	26·9	17·3	13·9	12·1	10·7	8·5	8·9

* Maximum reacting H-2 specificities: 1, 3, 8, 11, 25 and 32.

TABLE 4

CYTOLYSIS OF CBA (H-2^k) LYMPH NODE CELLS BY 1·5-FOLD SERIAL DILUTIONS OF HYPERIMMUNE C57 (H-2^b) ANTI-BP8 (H-2^k SARCOMA)* SERUM IN THE PRESENCE OF EXCESS RABBIT COMPLEMENT

Experiment No.	Per cent of total ^{51}Cr liberated by serum diluted:									Without serum with C′	Without serum or C′
	1 : 100	1 : 150	1 : 225	1 : 338	1 : 507	1 : 760	1 : 1140	1 : 1710	1 : 2565		
1	63	61	63	60	50	37	23	20	14	12	9
2	63	62	56	56	46	36	22	17	13	10	8
3	62	61	59	56	48	39	23	17	14	11	7
4	69	61	59	57	49	38	28	16	17	11	9
5	66	64	63	60	51	35	24	17	16	14	
6	64	63	61	58	53	41	26	18	15	11	
Arithmetic average	64·5	62	60	58	49·5	37·5	24·5	17·5	15	11·5	8

* Maximum reacting H-2 specificities: 1, 3, 8, 11, 25 and 32.

Results from sextuplicate titrations of two sera using both guinea-pig and rabbit complement are presented in Tables 1–4 and Figs. 1 and 2.

In the presence of rabbit complement, a smaller amount of isoantibody was required to kill a given number of target cells than in the presence of guinea-pig complement. In both cases, the slope of the antibody dilution curve was more shallow in the presence of rabbit complement than in the presence of guinea-pig complement. These two statements hold true for every mouse iso-antiserum which we have so far examined in this system.

The following experiments were done to elucidate the mechanism underlying the differences.

EFFECT OF ADDING HETEROANTIBODY AGAINST MOUSE GLOBULIN

To guinea-pig complement

We examined the possibility that the higher cytolytic efficiency of mouse isoantibody in the presence of rabbit complement was due to a 'sandwich' effect brought about by a natural antibody to mouse globulin present in the rabbit serum.

Four antisera, prepared in goats or rabbits, against whole mouse serum globulin or against mouse γ-globulin, were added at various dilutions to guinea-pig serum used as a source of complement in a standard isoantibody titration. One of the sera was without effect, whilst the other three gave consistent results. Data from one experiment are shown in Fig. 3. High concentrations of the 'sandwich' antibody lowered the apparent endpoint of the isoantibody titration, presumably due to competition for complement. For each antiglobulin serum, however, there was a certain optimal dilution which caused a shift to

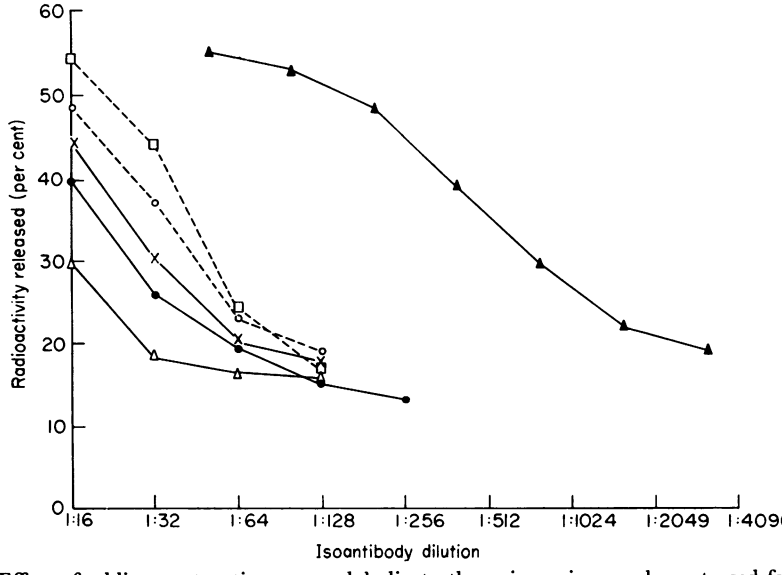

FIG. 3. Effect of adding goat anti-mouse-γ-globulin to the guinea-pig complement used for titration of H-2 cytolytic activity. Various concentrations (□, 1 : 1000; ○, 1 : 300, ×, 1 : 10,000; ●, nil; △, 1 : 100) of anti-globulin were added to 50 per cent guinea-pig serum. One volume of supplemented guinea-pig serum was added to mixtures of CBA (H-2^k) anti-EL4 (H-2^b lymphoma) serial dilutions and HTG (H-2^g) lymph node cells (10^7/ml) labelled with ^{51}Cr. The curve obtained when 25 per cent absorbed rabbit serum was used as a source of complement is shown for comparison (▲). Controls are mixtures from which isoantiserum was omitted.

the right, indicating an increased cytolytic efficiency of the mouse isoantibody. In no case did the increase equal that caused by the use of rabbit complement. The greatest increase in cytolytic efficiency due to added antiglobulin was about two-fold, very much less than the sixteen-fold increase seen in the presence of rabbit complement.

To rabbit complement

The same four antiglobulin sera used in the experiments described above were added, at various dilutions, to rabbit serum to be used as a complement source. The same one serum as was ineffectual when added to guinea-pig complement was also without effect in this system. The other three sera gave consistent results. Data from one experiment are shown in Fig. 4. In no case was the isoantibody dilution curve shifted to the right of that seen with rabbit complement alone, by the addition of antiglobulin. High concentrations of antiglobulin caused a marked shift to the left. This shift was decreased as the antiglobulin concentration was lowered, until the isoantibody dilution curve approached that seen when unadulterated rabbit serum was used as complement source.

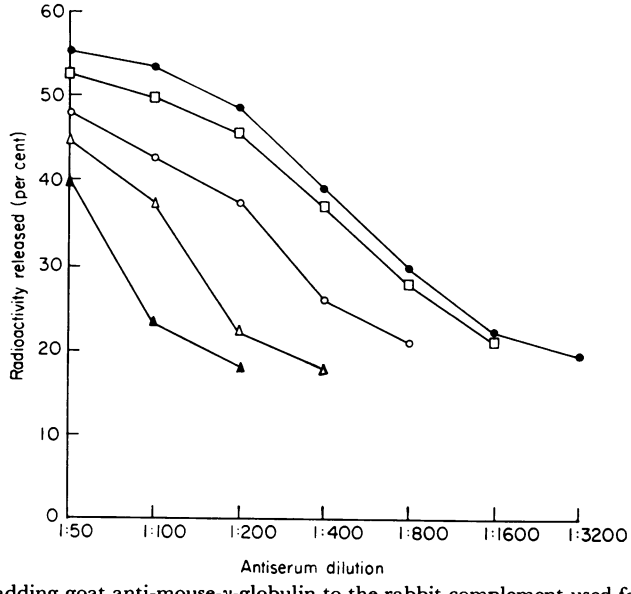

FIG. 4. Effect of adding goat anti-mouse-γ-globulin to the rabbit complement used for titration of H-2 cytolytic activity. Various concentrations (●, nil; ▲, 1 : 30; △, 1 : 100; ○, 1 : 300; □, 1 : 1000) were added to 25 per cent absorbed rabbit serum. Other details are as in Fig. 3.

HETEROANTIBODY DILUTION CURVES USING SHEEP ERYTHROCYTES AS TARGETS

Much of the information on complement kinetics is derived from studies of lysis of sheep erythrocytes with rabbit antibody. We questioned whether our peculiar findings were due either to the fact that we were studying mouse antibody or to the fact that we were not studying sheep erythrocytes. Accordingly, CBA mice were hyperimmunized with sheep erythrocytes following a schedule similar to that employed in the production of our CBA anti-EL4 antiserum. This antiserum was then titrated against ^{51}Cr-labelled 1 per cent (v/v) sheep red cells in the presence of either guinea-pig or rabbit complement. Results

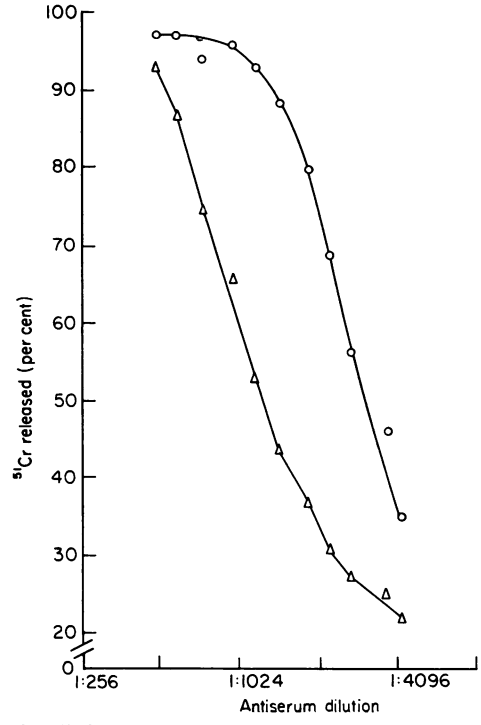

FIG. 5. Haemolysis of ^{51}Cr-labelled sheep red blood cells by dilutions of a hyperimmune CBA antiserum in the presence of excess guinea-pig or rabbit complement. The points plotted are the arithmetic averages of quadruplicate determinations. Serum: CBA-SRBC; cell: 1 per cent SRBCs; complement: RC′ 1 : 4 (△), GPC′ 1 : 2 (○). Control: △ + RC′ 1 : 4, ○ + GPC 1 : 2.

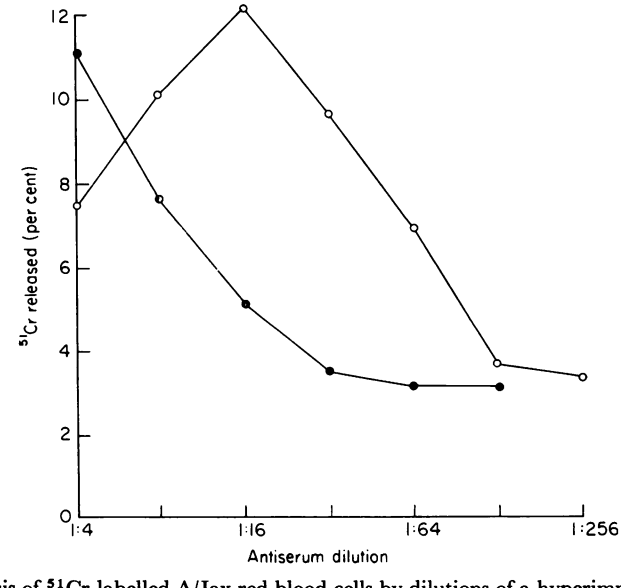

FIG. 6. Haemolysis of ^{51}Cr-labelled A/Jax red blood cells by dilutions of a hyperimmune (C3H × C57) anti-A/Jax serum, in the presence of excess guinea-pig (●) or rabbit (○) complement. The points plotted are the arithmetic averages of triplicate determinations.

of one such titration are shown in Fig. 5. In contrast to the data obtained with isoantibody, the lytic efficiency of this antiserum was always higher in the presence of guinea-pig complement than in the presence of rabbit complement. The slopes of the dilution curves of heteroantibody were similar whether guinea-pig or rabbit complement was used.

We concluded that our findings in the isoimmune system were not due solely to a peculiarity of mouse antibody.

ISOANTIBODY DILUTION CURVES USING MOUSE ERYTHROCYTES

A hyperimmune antiserum prepared in (C3H × C57BL)F_1 by immunization with A/Jax spleen was titrated in the presence of excess guinea-pig or rabbit complement, against 1 per cent A/Jax erythrocytes labelled with ^{51}Cr.

Results are shown in Fig. 6. We were unable to get better than about 10 per cent lysis in this system and the dilution curve with rabbit complement showed a marked prozone. In view of this, the data cannot be interpreted in terms of slope, but it was clear that the isoantibody gave more efficient lysis of erythrocytes in the presence of rabbit than of guinea-pig complement.

COMPLEMENT TITRATIONS

The complement activity of guinea-pig and of rabbit serum was titrated in the presence of excess antibody in both the isoimmune and the heteroimmune systems.

In the isoimmune system, HTG lymph node cells and CBA anti-EL4 diluted 1 : 15 were used with added complement dilutions ranging from 1 : 2 to 1 : 32. In the heteroimmune system, 1 per cent (v/v) sheep erythrocytes and CBA antibody diluted 1 : 500 were used, with complement dilutions ranging from 1 : 2 to 1 : 256. Data obtained in the two systems are presented in Table 5. A greater amount of rabbit complement than of guinea-pig complement was required to lyse 50 per cent of the sheep cells, whereas the opposite was true in the isoimmune system. It is clear that in comparing different sera with respect to complement activity, it is meaningless to discuss 'amount' of complement contained without reference to the system being used for measurement.

TABLE 5

COMPLEMENT ACTIVITY OF GUINEA-PIG AND OF RABBIT SERUM IN THE PRESENCE OF EXCESS MOUSE (CBA) ALLOANTIBODY OR HETEROANTIBODY

Antiserum	Target cells	Dilution of complement giving 50 per cent lysis	
		Guinea-pig	Rabbit
Anti-SRBC 1 : 500	Sheep red blood cells	1 : 50	1 : 16
Anti-H-2 1 : 15	HTG lymph node cells	1 : 6	1 : 16

COMPARISON OF MERCAPTOETHANOL SENSITIVE AND RESISTANT ANTIBODIES

It has been reported that guinea-pig complement reacts more efficiently with mouse 19S antibody and rabbit complement more efficiently with mouse 7S antibody (Winn, 1965).

TABLE 6

CYTOTOXIC UNITS OF ANTIBODY PRESENT IN AN ANTISERUM (C57-HTH) FROM AN EARLY BLEEDING USING RABBIT COMPLEMENT AND GUINEA-PIG COMPLEMENT IN THE TITRATION

	Antibody cytotoxic units (1/antiserum dilution)		
	Total	Mercaptoethanol resistant	Mercaptoethanol sensitive
Guinea-pig complement (GPC′)	56	8	48
Rabbit complement (RC′)	224	54	170
Ratio: RC′/GPC′	4·0	6·75	3·54

An antiserum was prepared in C57BL/10 mice by immunization with HTH tissues according to a schedule calculated to produce mainly 19S antibody. This was titrated against HTH lymph node cells, both before and after treatment with 0·1 M 2-mercaptoethanol, in the presence of excess guinea-pig or rabbit complement. Fifty per cent cytolytic endpoints of the four titrations are presented in Table 6. Units of mercaptoethanol sensitive antibody were calculated by subtracting mercaptoethanol resistant units from the total. Both classes of antibody showed a higher cytolytic efficiency in the presence of rabbit complement than of guinea-pig complement, although the difference was greater in the case of mercaptoethanol resistant antibodies.

DISCUSSION

Immune cytolysis results from the activation of serum complement by IgM or IgG antibody molecules specifically combined with antigens at the cell surface. Activation of complement is believed to involve a series of sequential steps involving at least nine different components and resulting in the local perforation or weakening of the cell membrane, thereby destroying its osmoregulatory characteristics (Green and Goldberg, 1960).

The kinetics of complement activation have been mainly studied in a single system. Sheep erythrocytes sensitized with heteroantibody (usually from rabbits) have been lysed with guinea-pig complement. It has been demonstrated that a single molecule of specifically combined IgM is adequate to activate complement, whereas two adjacent molecules of IgG are required (Humphrey and Dourmashkin, 1965; Borsos and Rapp, 1965). Thus, IgM has a much greater haemolytic efficiency than IgG. This is true also for mouse antibody against sheep erythrocytes (Wigzell, Möller and Andersson, 1966). The haemolytic efficiency of IgG may be increased by adding antiglobulin to the reaction system (Wigzell, 1966), thus providing the basis for the enumeration of IgG producing cells in the 'indirect' Jerne technique (Sterzl and Riha, 1965).

Analyses of antibody dilution curves for IgG and IgM anti-sheep red cell antibody, by the method of von Krogh (1916) or Weinrach, Lai and Talmage (1958) give apparent orders of reaction close to 2 and close to 1, respectively. This, together with C′1 transfer studies, is the basis for believing that molecular doublets of IgG are required for activation of complement, whereas single molecules of IgM are adequate.

Similar analyses have been reported of antibody dilution curves, using mouse IgG and IgM directed against antigens of the H-2 system, nucleated target cells and guinea-pig complement (Andersson, Wigzell and Klein, 1967). Apparent orders of reaction of close to 2 and close to 1, respectively, have been interpreted to mean that, in this system also, doublets of IgG or single molecules of IgM can activate complement.

We have found, in common with others, that rabbit complement behaves in a similar way to guinea-pig complement in a lytic system of sheep erythrocytes and heterologous antibody (Fig. 5). However, in an isoimmune cytolytic system, using hyperimmune antibody directed against H-2 antigens and nucleated lymph node cells, guinea-pig and rabbit complement behave quite differently (Figs. 1 and 2). In the presence of rabbit complement, a smaller amount of antibody is required to lyse 50 per cent of the target cells, and the slope of the antibody dilution curve is less steep. This seems not to be due to the presence of a natural antiglobulin in rabbit serum. The addition of anti-mouse-globulin to guinea-pig complement caused a modest shift of the antibody dilution curve to the right but this did not approach the curve seen when rabbit complement was used (Fig. 3). Furthermore, addition of antiglobulin to rabbit complement could only decrease the apparent cytolytic efficiency of the mouse isoantibody (Fig. 4), shifting the dilution curve to the left.

TABLE 7

APPARENT ORDERS OF REACTION OBTAINED GRAPHICALLY FROM WEINRACH AND VON KROGH PLOTS, USING GUINEA-PIG COMPLEMENT AND RABBIT COMPLEMENT

Weinrach		von Krogh	
GPC′	RC′	GPC	RC′
H-2 Cytotoxic antisera			
CBA-EL4			
1·63	0·73	2·59	0·82
1·82	0·95	2·50	1·14
2·29	0·78	2·56	1·50
2·79	1·00	2·82	1·15
——	1·00	——	1·14
2·13*	——	2·62*	1·33
	0·89*		——
			1·18*
Ratio GPC′/RC′ = 2·40		Ratio GPC′/RC′ = 2·22	
C57-BP8			
3·07	1·85	1·45	0·67
3·00	1·75	4·25	2·30
——	——	3·95	2·30
3·04*	1·80*	——	——
		3·22*	1·76*
Ratio GPC′/RC′ = 1·69		Ratio GPC′/RC′ = 1·83	
Average for two H-2 antisera Ratio GPC′/RC′ = 2·05		Average for two H-2 antisera Ratio GPC′/RC′ = 2·02	
Heterologous antiserum			
CBA-SRBC			
1·82	1·98	2·86	3·83
2·64	2·27	3·00	2·60
——	——	——	——
2·23*	2·13*	2·93*	3·21*
Ratio GPC′/RC′ = 1·05		Ratio GPC′/RC′ = 0·91	

* Mean values.

We have analysed a series of replicate H-2 antibody dilution curves, using two different antisera and using either guinea-pig or rabbit complement, by the methods of von Krogh and of Weinrach. Apparent orders of reaction are shown in Table 7, together with similar analyses of sheep cell haemolysis. In the H-2 system, the apparent orders of reaction obtained with guinea-pig complement were consistently higher than those obtained with rabbit complement. The average ratio of orders of reaction, GPC′ : RC′, was close to 2 : 1. This could be interpreted to mean that a single molecule of fixed antibody is able

to activate rabbit complement, whereas a molecular doublet is required for activation of guinea-pig complement. It does not, however, hold good for lysis of sheep erythrocytes where guinea-pig and rabbit complement give approximately equal apparent orders of reaction. The interpretation would imply a specificity of complement for the target cells.

A number of other differences exist between isoimmune lysis of lymph node cells and heteroimmune lysis of sheep erythrocytes. A very much higher concentration of guinea-pig serum is required to complement the former system than the latter, whereas a similar concentration of rabbit serum suffices in the two (Table 5). In the sheep cell system, there are about 7×10^5 antibody combining sites per cell, whereas in the H-2 system the number is at least 100 times lower (Haughton and Nash, unpublished data). Sheep erythrocytes are notoriously fragile, whereas mouse lymph node cells are relatively tough. Maybe a repair mechanism exists at the lymph node cell membrane which is absent from sheep erythrocytes and that the repair mechanism is more effective against damage inflicted by activated guinea-pig complement than against activated rabbit complement. The data only show that a greater number of simultaneous events are required at some stage leading to lysis by guinea-pig complement. This interpretation is supported by the finding that mercaptoethanol sensitive isoantibody (presumed to be IgM) shows a higher cytolytic efficiency with rabbit complement than with guinea-pig complement (Table 6). If a single molecule of IgM be sufficient to activate complement, then activated guinea-pig complement must be less efficiently lytic than activated rabbit complement.

Further studies will be necessary to define the exact nature of the difference between rabbit and guinea-pig complement in isoimmune cytolysis. We do not propose to undertake these studies. Our objective was to increase the sensitivity of the ^{51}Cr monitored cytolytic method for measuring mouse histocompatibility antibody, without sacrificing the precision inherent in the technique. We feel that we have accomplished this, have defined the precautions to be taken in using rabbit complement and have described the major characteristics of isoimmune lysis in the presence of rabbit complement. We are now more interested in applying the higher sensitivity of the modified method to studies of histocompatibility, than in studying the method itself.

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