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Autoantibody Production in Rabbits

V. COMPARISON OF THE AUTOANTIBODY RESPONSE AFTER THE INJECTION OF RAT AND RABBIT LIVER AND BRAIN

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Summary. The injection of rat liver and rat brain in Freund's complete adjuvant into rabbits led to the production of complement fixing autoantibodies against rabbit tissue. The autoantibody response following the injection of rabbit liver and brain was much smaller.

The autoantibody response to rat liver occurred even when Freund's incomplete adjuvant was used. The injection of rabbit liver in Freund's complete adjuvant into rabbits which had produced autoantibody after the injection of rat liver did not increase the level of autoantibody to rabbit liver.

In most, but not all, rabbits the natural antibody found in the sera taken before immunization was destroyed by heating the sera at 65°. After immunization with rat liver in Freund's complete adjuvant the levels of serum antibody to rabbit liver stable at 56° rose by day 5; however, autoantibody stable at 65° was not detectable until between days 7 and 14.

INTRODUCTION

The injection of various tissues from the rat and other species into the rabbit produces serum factors which react with rabbit tissue in the complement fixation, gel diffusion and immunofluorescence tests and behave like autoantibodies (see Asherson and Dumonde, 1963; Johnson, Asherson, Kaklamonis and Dumonde, 1963). Autoantibody production against rabbit glutamic dehydrogenase has also been demonstrated after the injection of crystalline bovine glutamic dehydrogenase (Bollet, Davis and Hurt, 1962). Kaplan (1958, 1960) showed that although the rabbit gives a good autoantibody response after injection of bovine heart, no antibody production follows the injection of rabbit heart. In the experiments of Gery and Davies (1961a, b) a few of the rabbits injected with rabbit heart produced antibodies to rabbit heart detectable by the tanned cell technique.

This paper provides evidence that rat liver and brain have a greater ability to cause autoantibody formation in the rabbit than the corresponding rabbit tissues.

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MATERIALS AND METHODS

Immunization of Rabbits

Sandy lop rabbits were used in the experiments on the response to injection of brain. Unless otherwise stated all other experiments were performed on Dutch rabbits bred in the Rheumatism Research Unit, Taplow. Fresh or freshly frozen organs from rabbits and rats were homogenized with an equal volume of 0·25 M sucrose using a Potter homogenizer with a teflon pestle and then emulsified with an equal volume of Freund's complete adjuvant (Difco). Brain was emulsified with Freund's incomplete adjuvant (Difco) to which 0·8 mg./ml. of *Mycobacterium tuberculosis* had been added. Each rabbit received 0·5 ml. of the emulsion of tissue homogenate and adjuvant in each of the four footpads. In some experiments rabbit liver was homogenized with an equal volume of rat serum instead of 0·25 M sucrose. In another experiment rat liver was homogenized with Freund's incomplete adjuvant (Difco). In the experiment on the response to a second injection of rabbit and rat tissue, footpad injections were given 53 days after the first injection.

Complement Fixation Test

The test antigens were homogenates of rabbit liver, kidney and brain and rat liver (one part of tissue by wet weight in 9 parts of 0·25 M sucrose) and were diluted on the day of use with calcium–magnesium–saline. The rabbit liver and kidney and rat liver were used at dilutions of 1/60 to 1/80 dilution wet weight/volume; the rabbit brain was diluted 1/200. Sera were heated at 56° for 30 minutes unless otherwise stated. In some experiments the sera were heated at 65°. Three MHD_{100} of complement were used and two volume antibody and antigen controls included. The titres are given as the reciprocal of the highest dilution of serum giving significant (2 plus) complement fixation in a system containing equal volumes of serum, complement, antigen and sensitized red cells. Other details have been given (Asherson and Dumonde, 1963).

Serum Glutamic Oxaloacetic Transaminase

The method and reagents described in Sigma technical Bulletin No. 505 (1961) were employed. The results were expressed in Sigma-Frankel units.

RESULTS

COMPARISON OF THE ABILITY OF RAT AND RABBIT LIVER TO CAUSE AUTOANTIBODY FORMATION

Twelve rabbits were immunized—three with rabbit liver in Freund's complete adjuvant, three with rabbit liver mixed with rat serum in Freund's complete adjuvant, two with rat liver in Freund's complete adjuvant, two with rat liver in Freund's incomplete adjuvant and two with Freund's complete adjuvant alone. The rabbits were bled daily or on alternate days for the first 10 days and at 14, 21 and 28 days after immunization. A rise in titre of two tubes (2 $\log_2$ units) was regarded as significant.

Neither of the rabbits which received Freund's complete adjuvant alone showed a rise in complement fixing antibody titre to rabbit liver, rabbit kidney or rat liver. Both rabbits which received rat liver in Freund's complete adjuvant showed a significant rise in titre to the three antigens by day 5. The two rabbits injected with rat liver in Freund's incomplete adjuvant showed a significant rise by day 6 (Table 1).

TABLE 1

TITRE OF COMPLEMENT FIXING ANTIBODY AGAINST RABBIT LIVER AND KIDNEY AND RAT LIVER AFTER ONE INJECTION OF RAT LIVER IN FREUND'S COMPLETE AND INCOMPLETE ADJUVANT

Day	*Rat liver in Freund's complete adjuvant*						*Rat liver in Freund's incomplete adjuvant*					
	Rabbit 1			*Rabbit* 2			*Rabbit* 3			*Rabbit* 4		
	RbL	*RbK*	*RtL*	*RbL*	*RbK*	*RtL*	*RbL*	*RbK*	*RtL*	*RbL*	*RbK*	*RtL*
0	8	16	8	8	16	8	16	32	16	16	64	16
3	8	16	8	8	16	8						
4	16	64	32	16	32	16	32	32	32	64	128	64
5	32	64	64	64	64	64						
6	64	128	128	32	64	64	64	128	64	256	256	256
7	64	128	128	64	64	64						
14	32	128	64	32	128	128	32	128	128	128	256	256
21	32	64	64	32	64	128	32	64	64	128	256	256
28	32	64	64	16	128	128	16	64	64	64	256	128

The test antigens are homogenates of rabbit liver (RbL), rabbit kidney (RbK) and rat liver (RtL). The results are expressed as the reciprocal of the highest dilution of serum giving significant complement fixation. The initial titres of two rabbits injected with Freund's complete adjuvant alone against the three antigens were 32, 64, 32 and 16, 64, 32, respectively. The titres of the first rabbit did not rise, those of the second rabbit rose to 32, 128, 32; 32, 128, 64 and 32, 64, 32 on days 14, 21 and 28, respectively.

On the other hand none of the six rabbits which received rabbit liver in Freund's complete adjuvant either with or without rat serum showed a significant rise in auto-antibody titre by day 6. One rabbit showed a transient rise on day 8 while three rabbits showed a significant rise at 14, 21 and/or 28 days. When the sera were inactivated at 65° instead of at 56° only one gave a titre greater than 4 and none of the sera from the six rabbits showed a significant rise in titre.

TABLE 2

MEAN AND RANGE OF THE TITRE OF COMPLEMENT FIXING ANTIBODY AGAINST RABBIT LIVER AFTER ONE INJECTION OF RAT OR RABBIT LIVER IN FREUND'S COMPLETE ADJUVANT EXPRESSED IN LOG_2 UNITS

Day	*Immunizing antigen—rat liver (five rabbits)*				*Immunizing antigen—rabbit liver (six rabbits)*			
	Serum heated at 56°		*Serum heated at* 65°		*Serum heated at* 56°		*Serum heated at* 65°	
	Mean titre	*Range*	*Mean titre*	*Range*	*Mean titre*	*Range*	*Mean titre*	*Range*
0	2·8	2–4	1·0	1	3·0	1–6	1·0	1
3	3·0	2–4	1·0	1	2·7	1–5	1·0	1
4	3·6	2–5	1·0	1	2·5	1–4	1·0	1
5	6·0	5–7	1·0	1–2	2·8	1–6	1·0	1
6	6·0	5–7	1·2	1–2	3·2	1–6	1·7	1–3
7	5·8	5–7	1·2	1–2	3·3	1–6	2·0	1–4
14	5·4	5–6	3·2	1–4	2·8*	1–6	1·7	1–4
21	4·8	4–6	4·4	3–5	2·8†	1–7	1·3	1–3
28	4·6	4–6	4·0	2–5	2·8†	1–7	1·3	1–3
35	4·4	4–5	3·0	2–4	2·8	1–6	1·0	1
53	3·8	3–4	2·2	2–3	2·3	1–5	1·0	1

For the purpose of this table a titre of <8, <4 and $\leqslant 8$ were regarded as 4, 2 and 8, respectively. The results are expressed as the mean of the log to base 2 of the reciprocal of the highest dilution of serum giving significant complement fixation. Thus figures of 5 and 6 mean that the highest dilutions giving significant complement fixation were 1/32 and 1/64, respectively.

* The values for two missing titrations were assumed.

† The value for one missing titration was assumed.

To test further the impression that rat liver was more effective in eliciting antibody formation than rabbit liver, six rabbits were injected with rat liver in Freund's complete adjuvant and six rabbits with rabbit liver in Freund's complete adjuvant. One of the rabbits receiving rat liver died. Microscopy did not show significant liver pathology. Following the injection of rat liver in adjuvant the autoantibody titre to rabbit liver and kidney increased on day 5, reached its highest level on day 6 and 7 and returned almost to its initial value by day 53. Antibody to rat liver also rose by day 4 and was still elevated on day 53.

TABLE 3

TITRE OF COMPLEMENT FIXING ANTIBODIES AGAINST RABBIT LIVER AND KIDNEY AND RAT LIVER AFTER AN INJECTION OF RAT LIVER IN FREUND'S COMPLETE ADJUVANT

Day	*Sera heated for 30 min.* (° *C.*)	*Serum 1*			*Serum 2*			*Serum 3*		
		Test antigens			*Test antigens*			*Test antigens*		
		RbL	*RbK*	*RtL*	*RbL*	*RbK*	*RtL*	*RbL*	*RbK*	*RtL*
0	56	<8	64	16	⩽8	64	16	16	64	16
	65	<4	<4	<4	<4	<4	<4	<4	<4	<4
3	56	<8	64	⩽16	16	64	16	⩽16	64	16
	65	<4	<4	<4	<4	<4	<4	<4	<4	<4
4	56	⩽16	128	64	⩽32	128	64	<16	64	16
	65	<4	<4	<4	<4	<4	<4	<4	<4	<4
5	56	128	512	128	64	256	128	64	128	128
	65	<4	4	4	<4	<4	4	<4	<4	<4
6	56	128	512	256	64	256	128	32	128	128
	65	4	4	32	<4	4	32	<4	<4	32
7	56	64	512	256	128	512	256	32	128	128
	65	4	4	32	<4	4	32	<4	<4	32
14	56	64	512	512	64	256	256	32	128	128
	65	16	64	64	16	128	128	8	32	128
21	56	64	512	512	32	512	256	32	128	128
	65	32	128	64	32	128	128	16	64	128
28	56	64	512	512	32	256	256	16	128	128
	65	32	128	64	16	128	128	16	64	128
35	56	32	256	128	32	256	256	16	128	128
	65	16	64	64	16	128	128	8	32	64
53	56	16	128	128	16	64	64	8	64	64
	65	4	32	32	8	32	64	4	16	32

The results of three of the five rabbits studied are presented. Rabbits 1 and 2 showed the highest rise in titres and Rabbit 3 the smallest rise. The test antigens were rabbit liver (RbL), rabbit kidney (RbK) and rat liver (RtL).

After inactivation at 65° the autoantibody titres before immunization were less than 4. Complement fixing activity to rat liver appeared by day 6. Autoantibody to rabbit liver and kidney was absent on day 7 but present on day 14. The highest level was on day 21 and raised titres were still present at day 53.

On the other hand none of the six rabbits injected with rabbit liver had a significant

rise of autoantibody titre after inactivation at 56°. The sera of three of the rabbits, however, showed prozones at high antibody concentration between days 14 and 35 which were not found in earlier bleeds. After inactivation at 65° the sera of three rabbits had low titres of autoantibody on day 7. In one of the rabbits autoantibody was also present on days 14, 21 and 28. These results are presented in Tables 2 and 3. It can be seen that a rise in a autoantibody titre over the initial value may be apparent when serum is heated at 65° but not when the serum is heated at 56°. This is illustrated by the titres on day 1 and day 28 of serum 3.

It was concluded that rat liver caused greater autoantibody production than rabbit liver. Such autoantibody production occurred irrespective of whether the rat liver was injected with complete or incomplete adjuvant and the titre of antibody stable at 65° rose more slowly than antibody stable at 56° but labile at 65°.

SPONTANEOUS FLUCTUATION IN THE LEVEL OF NATURAL AUTOANTIBODY

The significance of the rise in titre in some of the rabbits receiving rabbit liver in Freund's complete adjuvant was difficult to assess. This is because spontaneous changes of the titre of natural autoantibodies occur in some batches of rabbits. Indeed in Sandy lop rabbits in another laboratory there was no clear-cut difference in the response to the injection of rabbit and rat liver in Freund's complete adjuvant. This was in part attributable to a poor antibody response and in part to fluctuation of the autoantibody titre of control rabbits. Such fluctuations were seen even after heating the sera at 65°. Similar fluctuation in the titre of natural autoantibody occasionally occurred in rabbits bred at Taplow.

Another example of the variation in the behaviour of rabbits was provided by eight rabbits purchased from a dealer. Even before immunization the complement fixing autoantibodies in the sera of these rabbits were heat stable at 65°, unlike those of the Taplow rabbits. The titres against rabbit kidney after heating the sera at 56° ranged from 32 to 128. After heating at 65° the titres ranged from 4 to 32 and the mean drop was only 2·25 $\log_2$ units.

EFFECT OF A SECOND INJECTION OF RAT AND RABBIT LIVER

The eleven rabbits which had received rat or rabbit liver in Freund's complete adjuvant were given a second injection of liver in adjuvant. The results of the four combinations tested were:

(a) *Rat liver followed by rabbit liver.* Neither rabbit showed a significant rise in autoantibody titre.

(b) *Rabbit liver followed by rat liver.* The three rabbits showed a significant rise in autoantibody titre.

(c) *Rat liver followed by rat liver.* Both rabbits showed a significant rise in autoantibody titre.

(d) *Rabbit liver followed by rabbit liver.* One of the three rabbits showed a significant rise in antibody on days 10 and 14. Another rabbit showed a significant rise in autoantibody stable at 65° on days 7 and 10.

SERUM GLUTAMIC OXALOACETIC TRANSAMINASE ACTIVITY AFTER INJECTION OF RABBIT AND RAT LIVER IN FREUND'S COMPLETE ADJUVANT

The investigation of liver damage following experimental procedures in rabbits is complicated by the occurrence of spontaneous liver lesions including cirrhosis which are usually attributed to coccidial infection (Moon, 1934). It was found that liver damage from carbon tetrachloride was associated with very high levels of serum glutamic oxaloacetic transaminase. The range of values in eleven normal rabbits was 17–78. If the extreme value was excluded the range was 17–34 and the mean was 21·4. The subcutaneous injection of 2–4 ml./kg. of carbon tetrachloride into three rabbits led to levels of over 300 at 24 hours. The levels were elevated at day 4 in the two surviving rabbits.

The level of this enzyme was used as an index of liver damage in the eleven rabbits which had received injections of rat or rabbit liver in Freund's complete adjuvant. One of the rabbits injected with rabbit liver was arbitrarily omitted from the statistical analysis because of a high initial level of enzyme, which subsequently fell. The enzyme levels before immunization did not differ in the two groups of five rabbits. However 14 days after injection the level in the rabbits which had received rat liver had risen from a mean value of 19·4 to 29·8. The rise was significant ($0{\cdot}02>P>0{\cdot}01$). On the other hand, the level in the rabbits injected with rabbit liver rose from a mean value of 23·4 to 25·8. This rise was not significant ($0{\cdot}6>P>0{\cdot}5$). There was no significant difference ($0{\cdot}1>P>0{\cdot}05$) in the mean of the rises in the two groups of rabbits. These figures provide only doubtful evidence that rat liver in Freund's complete adjuvant causes a greater rise in serum glutamic oxaloacetic transaminase than rabbit liver under the same conditions.

COMPARISON OF THE ABILITY OF RAT AND RABBIT BRAIN TO CAUSE AUTOANTIBODY FORMATION

Three rabbits received rat brain and three rabbits received rabbit brain in Freund's complete adjuvant. Table 4 shows that a significant rise in autoantibody against rabbit brain occurred within 10 days in all three rabbits injected with rat brain but in none of the rabbits injected with rabbit brain. These figures were obtained using unheated serum

TABLE 4

TITRE OF COMPLEMENT FIXING ANTIBODIES AGAINST RABBIT BRAIN AND KIDNEY FOLLOWING THE INJECTION OF RABBIT AND RAT BRAIN IN FREUND'S COMPLETE ADJUVANT

Day	*Rabbits immunized with rabbit brain*						*Rabbits immunized with rat brain*					
	1		2		3		4		5		6	
	RbB	*RbK*	*RbB*	*RbK*	*RbB*	*RbK*	*RbB*	*RbK*	*RbB*	*RbK*	*RbB*	*RbK*
0	16	64	16	64	16	16	8	32	8	16	32	64
4	16	64	16	64	16	16	16	32	8	16	16	32
5	32	64	32	64	16	16	32	64	16	16	32	64
6	32	64	16	64	16	16	32	64	16	16	32	64
7	16	64	32	128	16	16	64	128	32	32	64	64
7*	4	4	<4	<4	<4	<4	8	4	4	<4	—	—
10	32	64	32	64	16	16	128	128	64	64	128	64
10*	4	<4	4	4	8	4	32	16	16	4	32	32

The test antigens are rabbit brain (RbB) and rabbit kidney (RbK).

* Sera heated at 65°. None of the sera taken before Day 7 had titres greater than 4 after heating at 65°. The other titres in this table were obtained with unheated sera.

but the pattern of the results on day 0 and day 10 was unaffected by heating the serum at 56°. After inactivation at 65° the sera of rabbits injected with rat brain showed a rise in titre of 3–4 $\log_2$ units while only one of the rabbits immunized with rabbit brain showed a rise as great as 2 $\log_2$ units. The rabbits were killed on day 10 and this may have forestalled a rise in titre in the other two rabbits. It was concluded that rat brain caused a greater autoantibody response than rabbit brain when injected into rabbits.

DISCUSSION

These results show that rat liver and brain have a greater ability than the corresponding rabbit organ to cause autoantibody formation in the rabbit. The injection of tissues of another species has been shown to be more effective in eliciting autoantibody production than that of tissues of the same species in the case of lens (Morax and Bollack, 1914; Halbert, Locatcher-Khorazo, Swick, Witmer, Seegal and Fitzgerald, 1957), uvea and cornea (Wacker and Dodd, 1961), brain (Lewis, 1933), heart (Kaplan, 1958, 1960; Gery and Davies, 1961a, b), adrenal (Barnett, Dumonde and Glynn, 1963), nuclear antigens (Goodman, 1959) and colon mucosa (Holborow, Asherson and Wigley, 1963). The behaviour of thyroid (Terplan, Witebsky, Rose, Paine and Egan, 1960; David and Holborow, 1961), red blood cells (Bussard and Hannoun, 1962) and testis is unclear.

The reason for the increased ability of tissue of other species (foreign tissue) to cause autoantibody production is unknown. Two experimental situations may be relevant:

i. Greater antibody production to lipid and simple chemical haptene occurs when the haptene is linked to a foreign carrier than when the haptene is linked to a carrier of the animal's own species (Landsteiner and Van der Scheer, 1925), and the ability of foreign carrier to enhance antibody production is diminished if the animal is made tolerant to the carrier (Cinader and Dubert, 1955; but see Boyden and Sorkin, 1962).
ii. Rabbits which are tolerant to bovine serum albumin produce antibody to bovine serum albumin when injected with human serum albumin and altered bovine serum albumin (Weigle, 1961, 1962).

In our experiment autoantibody increased within 5 days of the injection of rat liver into rabbits and the rabbits did not respond to a further injection of rabbit liver by producing increased levels of circulating autoantibodies. These facts are against the view that the injection of rat liver leads to the development by mutation of a clone of cells capable of responding to the injection of rabbit liver by the production of autoantibody.

It has often been suggested that the rabbit does not show immune tolerance to certain of its own brain components because the brain of young rabbits is deficient in the antigen with which the complement fixing antibody reacts (Schwentker and Rivers, 1934) and is secluded from the antibody producing system. This view however, leaves unexplained the poor autoantibody response following the injection of rabbit brain. Schwentker and Rivers (1934) showed that the ability of rabbit brain to cause the formation of complement fixing autoantibody in the rabbit was increased by adding foreign serum. It may be that rabbit brain is a poor immunizing antigen because it consists of determinants linked to native rabbit protein (to which the rabbit is tolerant) while rat brain is a good antigen because it consists of a similar haptene linked to a foreign protein (to which the rabbit is not tolerant).

The ability to produce autoantibody may not always be harmful to an animal and may, in some circumstances, be advantageous. Rowley and Jenkin (1962) proposed that one of the factors which limits host resistance to micro-organisms is the similarity of the surface antigens of the host's cells to those of the micro-organisms. The ability of foreign tissue to cause autoantibody formation under certain circumstances may serve to limit the occasions on which the host fails to give an antibody response to invading organisms. Indeed evolutionary pressure may have determined the optimal solution to the problem of balancing the risk of autoimmune disease against the risk of failing to give an immunological response against micro-organisms with antigens similar to self.

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