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DIFFERENCES IN IMMUNE ELIMINATION IN INBRED MICE

THE ROLE OF LOW AFFINITY ANTIBODY

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SUMMARY

The rate of elimination of soluble protein antigen injected intravenously differs in different inbred strains of mice previously immunized to the antigen. This difference is apparently related to the affinity of the antibody they produce. Animals producing high affinity antibody eliminate antigen more effectively than those producing low affinity antibody. Passive transfer of antibody shows that the difference of antigen elimination is a property of the antibody, rather than the cellular mechanisms involved. The immunopathological significance of these findings is discussed.

INTRODUCTION

Several studies over the past few years have shown that circulating soluble immune complexes are involved in the pathological processes leading to many diseases in man such as progressive nephritis (Unanue & Dixon, 1967). The best model of this type of disease is that of chronic serum sickness induced in a minority of rabbits by daily immunization with soluble protein antigens. Quantitative differences (Dixon, Feldman & Vazquez, 1961) and qualitative differences (Pincus, Haberkern & Christian, 1968; Christian, 1969; 1970) in the immune responses in these animals have been suggested as explanations for the differences in susceptibility to nephritis.

Oldstone & Dixon (1969) have shown that some inbred mice neonatally infected with lymphocytic choriomeningitis virus (LCM) get nephritis as a result of this infection while others do not. Previous work in this laboratory has suggested that susceptible strains produce antibodies of low affinity which may be poor at immune elimination of antigens so that circulating soluble immune complexes are formed. Any high affinity antibody produced by these animals would be in constant antigen excess, because of this failure. The resulting complexes formed in antigen excess would be responsible for the pathological lesions seen

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in these individuals (Soothill & Steward, 1971). We present evidence in this paper that low affinity antibody is indeed poor at immune elimination.

MATERIALS AND METHODS

Animals

The following inbred strains of mice, maintained at the Institute of Child Health, were used: Ajax, Simpson, C_3H, B10D2 new, C_{57} Black, SWR/J and CBA.

Experimental mice were 8–12-weeks old and weighed between 25–35 g. In each experiment, control groups were matched for age, sex and weight.

Antigens

Human serum albumin (HSA) was kindly provided by the Lister Institute of Preventive Medicine. Before use it was passed through a Sephadex G-200 column, and the fractions containing the peak concentration of albumin were pooled. ^{125}I-HSA was prepared by the iodine monochloride method (McFarlane, 1958).

Antibody preparations

Rabbit anti-HSA antiserum was obtained from an animal which had received a primary injection of HSA in Freund's complete adjuvant and had been boosted with alum precipitated antigen. Ajax, CBA and Simpson mice were immunized with 1 mg HSA in 0·1 ml saline intraperitoneally once a week for 4 weeks, and were bled by cardiac puncture under anaesthesia, 2 weeks after the last injection. In some experiments, globulin prepared by ammonium sulphate precipitation of the sera was used. B10D2 new mice were immunized by four-weekly injections of HSA in Freund's complete adjuvant.

Immune elimination by immunized animals

Mice were immunized with 1 mg HSA in saline intra-peritoneally weekly for 4 weeks, and were studied 2 weeks after the final injection. Twenty-four hours prior to the elimination experiments, they were given 0·2% KI in the drinking water to block thyroid uptake of radio-iodine. The immunized and matched control mice were injected with ^{125}I-HSA in 0·5 ml saline via the tail vein. Tail bleedings into 10 μl capillary tubes (Drummond Microcaps) were performed at intervals over a period of 30 hr following antigen administration. The blood samples were counted in a Panax gamma 160 spectrometer.

Immune elimination by passively transferred antibody

Groups of unimmunized mice, matched for age, sex and weight were given 0·2% KI in their drinking water. Experimental mice received mouse anti-HSA serum or globulin of known relative affinity and antibody content Ab_t (see below) via tail vein in a volume of 0·5 ml. Control mice received 0·5 ml unimmunized mouse serum. After 30 min all mice were injected via the tail vein with ^{125}I-HSA and elimination of antigen was followed by tail bleedings at intervals for a period of 6 hr.

Relative affinity measurements

The relative affinity and amount of antibody (Ab_t) of mouse anti-HSA antibodies in

serum and globulin fractions were measured as described by Steward & Petty (1972a, b). Ab_t is the amount of antibody expressed as $\mu\mu$moles antigen binding sites (Nisonoff & Pressman, 1953).

Measurement of globulin-bound antigen

The proportion of antigen remaining in the serum in a globulin-bound form during immune elimination was determined by precipitation with 50% saturated ammonium sulphate (Farr, 1958).

RESULTS

Elimination of antigen by immunized animals

Immunized animals of the various strains were given ^{125}I-HSA intravenously and the

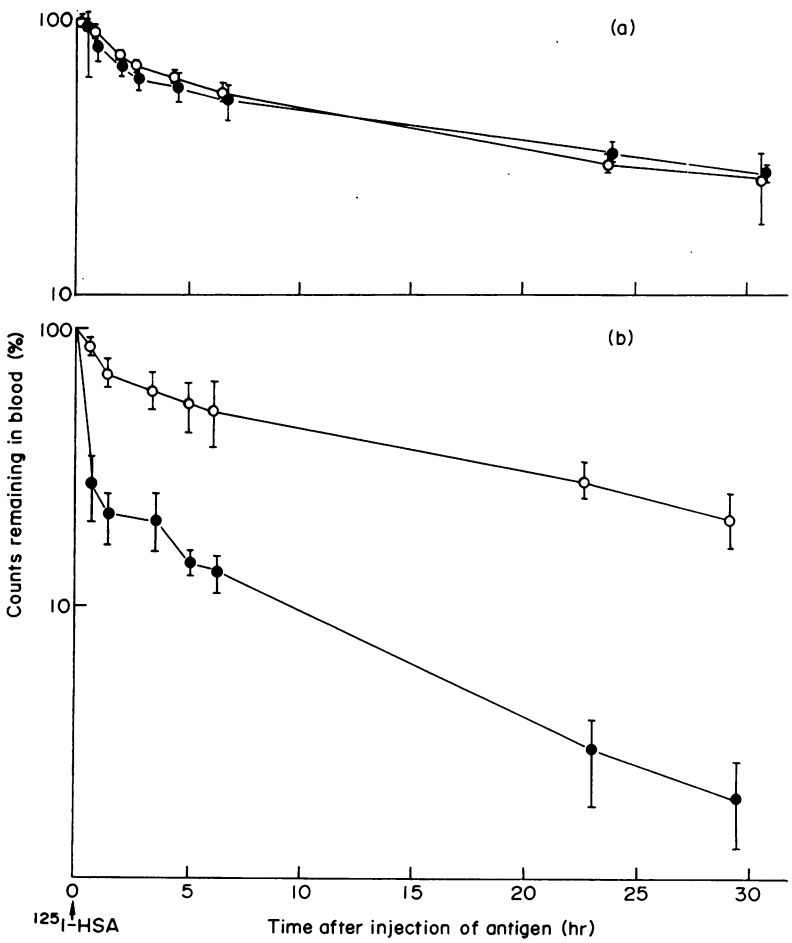

FIG. 1. Elimination by immunized mice of B10D2 new strain (a), and Ajax (b) of 50 μg ^{125}I-HSA given IV ●—●, Immunized mice; ○—○ unimmunized mice. Each curve represents the mean values from groups of three mice. The brackets indicate the observed range.

rate of disappearance of radioactivity from the blood followed. Representative elimination experiments in mice from two strains known to produce high (Ajax) and low (B10D2 new) affinity antibody (Petty, Steward & Soothill, 1972) are shown in Fig. 1. Ajax clearly are more efficient at eliminating antigen than B10D2 new. The data from all such experiments in seven strains of mice, using various doses of antigens are shown in Table 1. Residual radioactivity is expressed as

$$100 \times \frac{\text{Counts remaining in immunized animals}}{\text{Counts remaining in unimmunized animals}}$$

at three arbitrarily selected times. A value less than 100 indicating that immune elimination had occurred was observed in all strains, though only with low antigen doses in B10D2 new and SWR/J which make low affinity antibody. We include values for relative affinity (K_R) and Ab_t ($\mu\mu$moles/ml) for mice of the same strains, published elsewhere (Petty *et al.*, 1972).

TABLE 1. Active immune elimination of ^{125}I-HSA by several strains of inbred mice immunized with HSA. Except where indicated (*) values are calculated from the mean of groups of three immunized and unimmunized mice

Mouse strain	Relative affinity L/M (log mean)	Ab_t $\mu\mu$moles/ml (log mean)	^{125}I-HSA injected (μg)	Counts remaining in immunized mice / Counts remaining in unimmunized mice × 100		
				10 min	1 hr	2 hr
Ajax	$2{\cdot}9 \times 10^6$	450	100	60	36	40
			50	32	31	35
Simpson	$2{\cdot}1 \times 10^6$	3400	200	68	33	24
			25	47	39	38
C_3H	$1{\cdot}2 \times 10^6$	312	100	67	57	62
			50	90	84	83
CBA	$3{\cdot}4 \times 10^5$	615	200	70	74	54
			25	77	86	73
C_{57}Bl	$2{\cdot}6 \times 10^5$	312	200	96	86	80
			25	91	82	77
SWR/J	$5{\cdot}7 \times 10^5$	1140	200*	95	92	106
			50*	91	84	75
			25*	84	84	80
B10D2 new	$3{\cdot}1 \times 10^5$	250	200	—	102	103
			50	98	97	95
			25	100	96	90
			12·5	79	75	72

* Two mice per group.

At high doses of antigen, strains of mice producing antibody of high relative affinity eliminated the antigen effectively, however much antibody they produced. The strains producing low affinity antibody achieved little or no elimination. There is some suggestion that the amount of antibody produced is also relevant, since CBA mice which produce relatively high levels of low affinity antibody, were intermediate in their capacity for immune

TABLE 2. Active immune elimination of 200 μg ^{125}I-HSA (injected IP) by mice immunized with HSA in Freund's complete adjuvant

Mouse strain	Number of animals		$\frac{\text{Counts remaining in immunized mice}}{\text{Counts remaining in unimmunized mice}} \times 100$	
	Immunized	Unimmunized	1 hr	2 hr
Ajax	3	3	50	15
B10D2 new	3	3	64	35

elimination. Our data suggest that, though both are relevant, immune elimination is less closely related to Ab_t of an animal's antibody response than to its affinity. For instance, Ajax mice which eliminate effectively, produce approximately one third as much anti-HSA antibody as SWR/J mice, which eliminate poorly. Similar doses of antigen (50 μg) were given to the mice illustrated in Fig. 1 in which a large difference in elimination is associated with only a two-fold difference in Ab_t. When the amount of antigen given to the B10D2 new mice is adjusted to equalize the Ab_t : antigen ratio to that in Ajax mice, the large difference in elimination remains. Presumably, the immune elimination observed in B10D2 new mice at low antigen doses (12·5 μg) is due to the fact that the Ab : Ag ratio is approaching antibody excess. Elimination of intraperitoneally injected labelled antigen (200 μg) in B10D2 new mice immunized with antigen in Freund's complete adjuvant was more effective than animals immunized with antigen in saline. These mice eliminated the antigen at a rate similar to that of Ajax mice treated in the same way (Table 2).

Elimination of antigen by passively transferred antibody

Preliminary passive transfer experiments using various relative concentrations of antigen and heterologous high affinity rabbit anti-HSA serum indicated the importance of the ratio of antibody to antigen in antigen elimination. The results of such experiments in two strains of mice, one good at active immune elimination (Ajax) and one poor at active

TABLE 3. Immune elimination of ^{125}I-HSA by inbred mice after the passive transfer of heterologous rabbit anti-HSA to give different antibody: antigen ratios. Values are calculated from the mean of groups of three control and experimental mice

Recipient strain	Antibody: antigen ratio	$\frac{\text{Counts remaining in experimental mice}}{\text{Counts remaining in control mice}} \times 100$		
		10 min	1 hr	2 hr
Ajax	1 : 4	97	92	94
	8 : 5	78	66	57
	7 : 1	56	21	24
B10D2 new	1 : 4	93	92	94
	8 : 5	81	64	62
	7 : 1	48	22	20

immune elimination (B10D2 new) are shown in Table 3. There was little or no difference in immune elimination of antigen in the two strains of recipient animals, at any of the antigen–antibody ratios used, but results differed markedly for the different ratios. The ratios were calculated from the Ab_t of the serum. This is the amount of antigen which a given volume of antiserum will bind, and so is expressed in terms of $\mu\mu$moles of antigen. Appropriate doses of antigen can therefore be expressed in the same units, as a ratio. For antigen excess experiments, the antigen dose was four times the Ab_t of the antiserum given, i.e. at an antibody-antigen ratio of 1 : 4. At such antigen excess there was little immune elimination. An antibody : antigen ratio of 7 : 1 produced a very rapid elimination of antigen. Antibody : antigen ratios approximating to equivalence (i.e. maximal precipitation in a precipitin test) for which the antibody:antigen ratio (based on Ab_t) was about 8 : 5 produced elimination approximately half-way between these extremes.

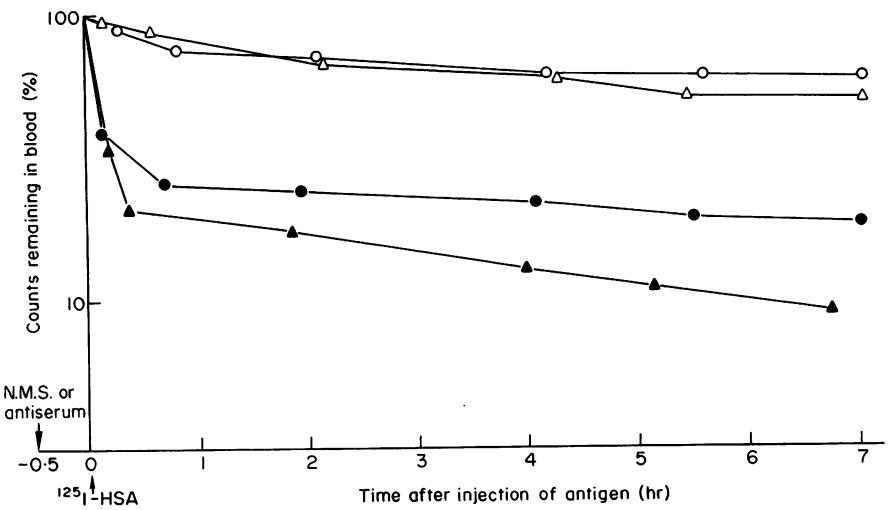

FIG. 2. Immune elimination by mice given homologous high affinity anti-HSA serum IV ●—●, B10D2 new mice given high affinity antibody from adjuvant immunized B10D2 new mice; ▲—▲, SWR/J mice given high affinity antibody from Ajax mice; ○—○, Control B10D2 new mice; △—△, control SWR/J mice. ^{125}I-HSA (50 μg) was given IV to all mice 30 min after the serum. Each point represents the mean value of groups of four mice.

Similar evidence of effective passive immune elimination of antigen by strains of mice defective in active immune elimination was obtained by passive transfer of homologous antibody of high affinity, followed by antigen at an antibody:antigen ratio of 8 : 5. Unimmunized B10D2 new mice received antibody from mice of the same strain immunized with antigen in adjuvant (Soothill & Steward, 1971). The SWR/J mice received antibody of high affinity from Ajax mice, immunized with antigen in saline. Both strains were able to eliminate the antigen effectively under these conditions (Fig. 2).

Experiments to compare the relative effect of passive transfer of antibody of different affinities are difficult to plan because of the effect of antibody:antigen ratios on immune elimination and the difficulty in defining similar amounts of antibody of different affinity. The fact that our low affinity antibody did not precipitate, also contributed to this problem.

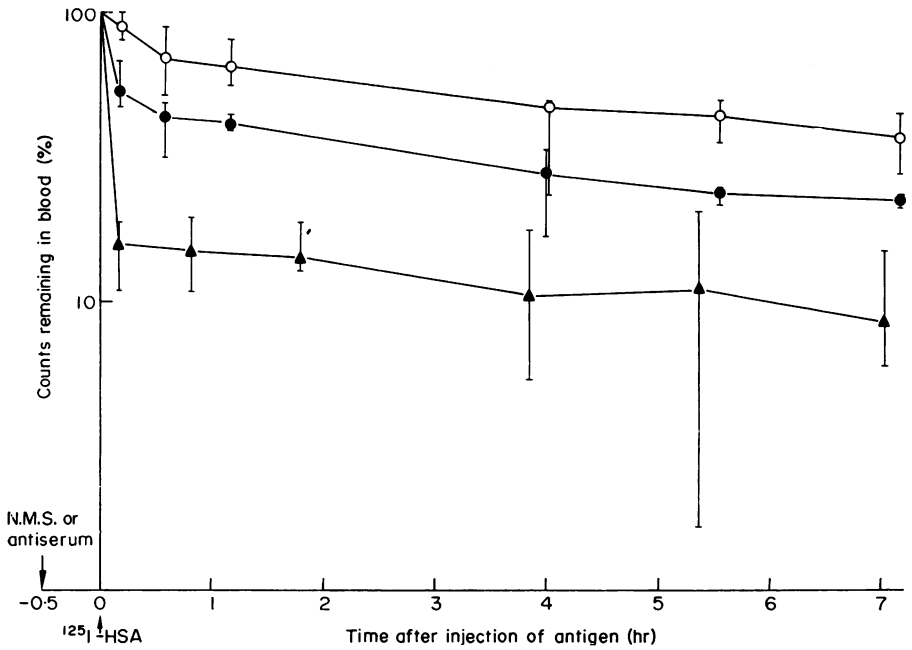

FIG. 3. Immune elimination by Simpson mice given equal amounts (IV) of anti-HSA (Ab_t = 560 $\mu\mu$moles) of high affinity (▲—▲) of low affinity (●—●). Control mice (○—○) were give normal mouse serum. ^{125}I-HSA (280 $\mu\mu$moles) was given IV to all mice 30 min after the serum. The mean value and range for groups of four mice are shown at each point.

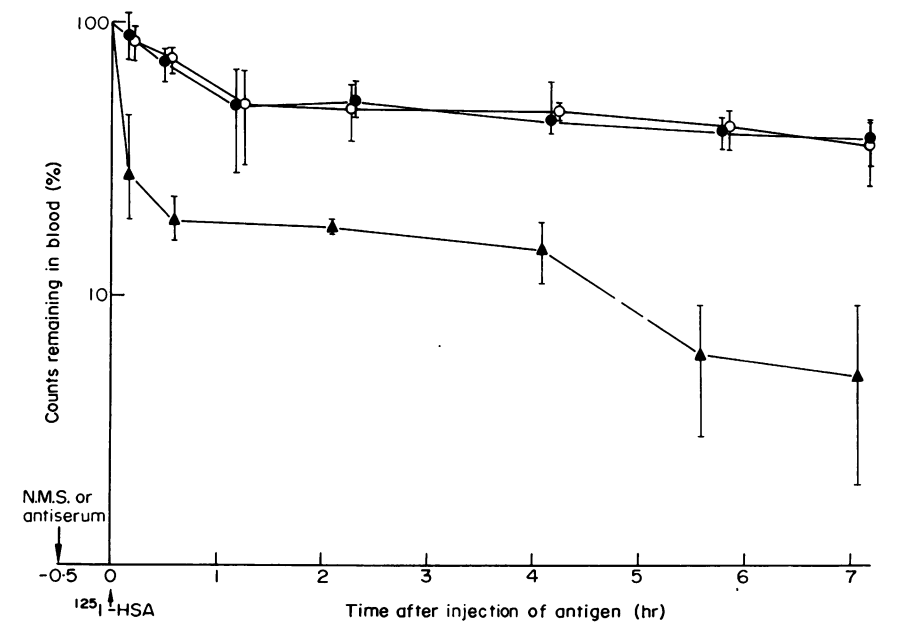

FIG. 4. Immune elimination by CBA mice given equal amounts (IV) of anti-HSA (Ab_t = 560 $\mu\mu$moles) of high affinity (▲—▲) or low affinity (●—●). Control mice were given normal mouse serum. ^{125}I HSA (280 $\mu\mu$moles) was given IV to all mice 30 min after the serum. The mean value and range for groups of four mice are shown at each point.

Two different approaches were used to establish the appropriate relative doses of antibody and antigen. First, a precipitin curve was constructed for mouse anti-HSA of high affinity of known Ab_t and equivalence was at an antibody:antigen ratio of 2 : 1 based on Ab_t. This ratio was used for this antibody and for a low affinity antibody in the following experiments to study immune elimination of antigen by high and low affinity antibody. Pooled sera from CBA mice (low affinity, $k_R = 1{\cdot}5 \times 10^5$ l/m) and Simpson mice (high affinity $k_R = 2{\cdot}3 \times 10^6$ l/m) immunized by antigen in saline, and globulin preparations from these sera were passively transferred into unimmunized mice of strains capable of making high or low affinity antibody. When the globulin preparations were passed through a Sephadex G-200 column and the first (19S) and second peaks (7S) pooled and concentrated, antibody binding of ^{125}I-HSA was only demonstrated in the second peak by ammonium sulphate globulin precipitation. Thus no demonstrable IgM antibody was present in the preparations. Representative results of these experiments are shown in Figs 3 and 4. In these experiments all mice received the same amount of antibody in terms of Ab_t units (560 $\mu\mu$moles) and the same amount of antigen (280 $\mu\mu$moles) to give a constant antibody:antigen ratio of 2 : 1. Antibody and antigen were injected in 0·5-ml volumes. High affinity serum was diluted in

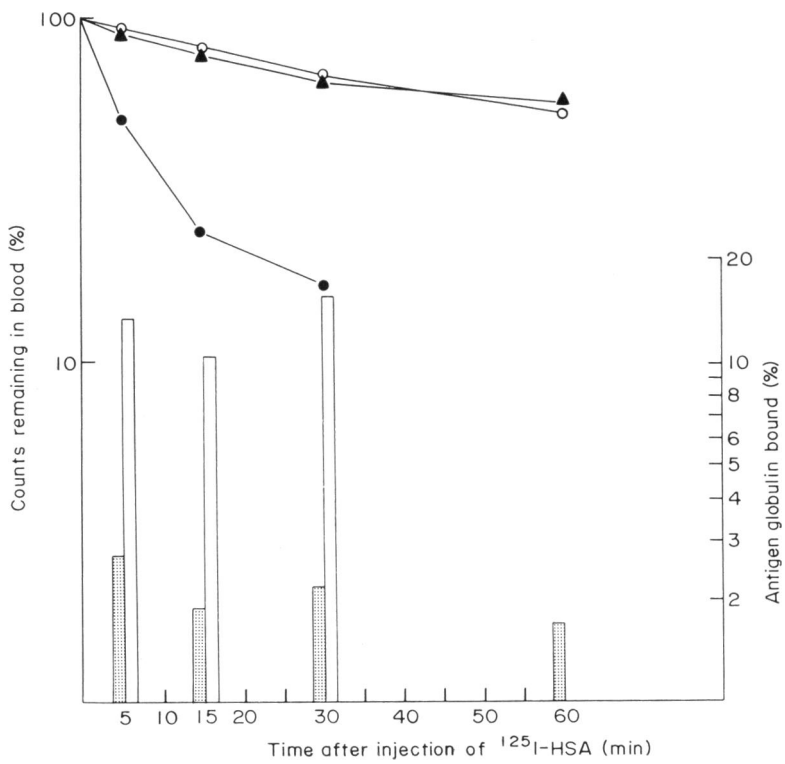

FIG. 5. Immune elimination by Ajax mice given equal amounts (IV) of anti-HSA globulin (Ab_t = 560 $\mu\mu$moles) of high affinity (●—●) or low affinity (▲—▲). Control mice (○—○) received normal serum. ^{125}I-HSA (280 $\mu\mu$moles) was given to all mice 30 min after the antibody. Each point is the mean of two mice. Globulin-bound antigen (corrected for non-specific binding) in mice given low affinity antibody shown by shaded columns; in mice given high affinity antibody, by open columns.

saline and low affinity serum concentrated by ultrafiltration. The data clearly demonstrate that unimmunized mice of both strains, when receiving high affinity antibody under these conditions, were capable of eliminating the antigen effectively. However, antigen elimination by the same strains given the low affinity antibody was much less. Therefore, the range of capacity for immune antigen elimination observed in the different strains mainly depends on the antibody produced or transferred, and is apparently related to its affinity. There is some suggestion that Simpson mice eliminate antigen complexed to low affinity antibody more rapidly than do CBA mice, although the latter handle high affinity antibody–antigen complexes as effectively as Simpsons.

Similar results were obtained when the globulin fractions of antisera of both high and low affinity were transferred passively at the same antibody:antigen ratio into unimmunized mice. Globulin-bound antigen was estimated in the sera by ammonium sulphate precipitation. As shown in Fig. 5 this value was far higher in those animals receiving high affinity antibody before the antigen than in those receiving the same amount of low affinity antibody. Thus in addition to having more antigen remaining, the latter had less of it in a globulin-bound form.

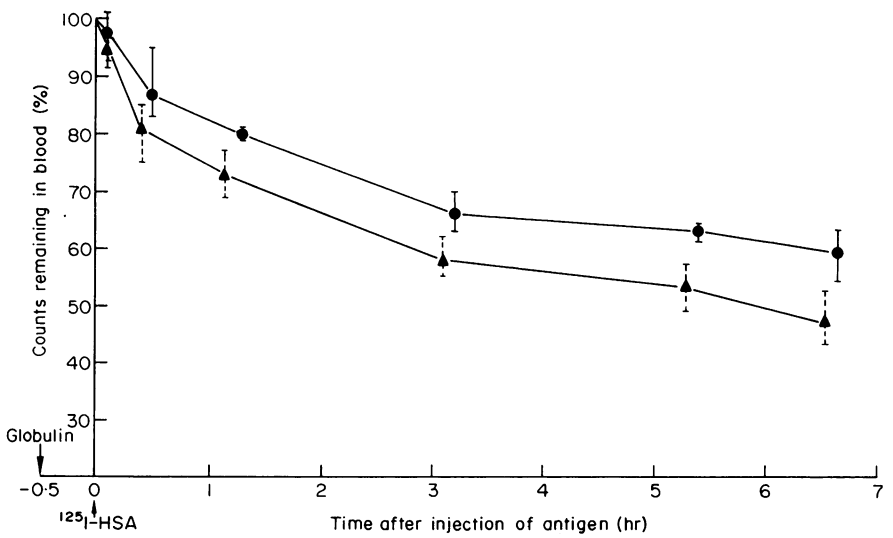

FIG. 6. Immune elimination by Ajax mice given globulin of high affinity (▲—▲) and low affinity (●—●) in amounts which bound 30% of the ^{125}I antigen (20 μg) *in vitro*. The mean value and range for groups of four mice are shown at each point.

The second approach for assessing the appropriate antibody dose for a given dose of antigen, using globulin preparations of different affinity, was based on their capacity to bind antigen *in vitro*. Volumes of globulin preparations from high and low affinity antisera which, when reacted *in vitro* with the dose of antigen used in these *in vivo* studies (20 μg) bound a similar proportion (30%) into an ammonium sulphate precipitable form, were used in a passive transfer experiment. There would clearly be more antibody molecules in such a volume of low affinity antibody globulin than of the high affinity antibody globulin

because of the greater dissociation rate of the former. Such amounts of Anti-HSA globulin of high or low affinity were injected IV into groups of four unimmunized mice prior to the injection of 20 μg of ^{125}I-HSA. The result of this experiment is shown in Fig. 6. At these doses, the high affinity antibody achieves immune elimination of antigen more rapidly than does the low affinity antibody. The differences are not as great as those in the previous experiments but a Rank Sum Test of the four values in each group at approximately 3 hr after injection of antigen, for example, showed the differences to be significant ($P<0{\cdot}05$). Since the experiments were carried out simultaneously, it was impossible to bleed the two groups of animals at the same time. Those animals receiving the high affinity antibody were bled first and the difference in time of the bleeds would therefore have the effect of reducing the differences between the two groups.

DISCUSSION

We have shown that different inbred strains of mice differ in their capacity to achieve immune elimination of antigen. Animals which are relatively poor at immune elimination after immunization by antigen in saline achieve good immune elimination after immunization by antigen in adjuvant. In general, the strains achieving good immune elimination of antigen were those producing high affinity antibody. Those that were poor at immune elimination produced low affinity antibody. Though there may also have been some relationship between capacity for immune elimination and Ab_t, it was less obvious.

Passive transfer experiments showed that this variation of function may be a property of the antibody produced by the different strains and is related to the relative affinity of the antibody (high affinity antibody is good at immune elimination and low affinity antibody is poor).

Factors possibly involved in the formation and clearance of immune complexes from the circulation include the following: the valency of the antigen (Lightfoot, Drusin & Christian, 1971); the antibody:antigen ratio (Lightfoot, Drusin & Christian, 1971; Mannik, Arend, Hall & Gilliand, 1971); the size of the complexes (Weigle, 1958; Benacerraf, Sebestyen and Cooper, 1959; Cochrane & Hawkins, 1969); complement and other serum factors (Maurer & Talmage, 1953; Paul & Benacerraf, 1965) and the functional integrity of the R.E.S. (Benacerraf, Sebestyen, & Cooper, 1959).

Much of the work on *in vivo* handling of antibody–antigen complexes has involved the use of isolated antibodies. By virtue of the methods involved in their isolation, therefore, most antibodies so far studied would be of relatively high affinity, and there is no data of the effect of antibody of low affinity. Since we have suggested that it may be of immunopathologic significance (Soothill & Steward, 1971), the role of low affinity antibody in immune elimination merits special consideration particularly if, for some reason, the proportion of such molecules is high. This may represent a qualitative defect in the immune response. The data presented in this paper indicate that the affinity of the antibody is also important in immune elimination, in addition to those factors listed above.

The different rates of immune elimination in the various strains observed in actively immunized animals are difficult to interpret since the dose of antigen given may have resulted in very different antibody:antigen ratios if one strain had produced more antibody than another. The passive transfer of serum experiments clearly showed that the strain differences observed are a property of the donor rather than the recipient. A range of

different antibody:antigen ratios was used in the experiments using rabbit antibody, and these confirmed that such ratios are critical. Assessing the appropriate dose of antibody of different affinity was difficult since the low affinity antibody did not precipitate. We therefore used the Ab_t term ($\mu\mu$moles of antigen-binding sites in the antibody preparation) as a measure of the amount of both high and low affinity antibody for calculating antibody: antigen ratios. In most of the experiments, equal amounts of high or low affinity antibody in terms of Ab_t were used at an antibody:antigen ratio of 2 : 1. Under these conditions, recipient mice were able to eliminate antigen more effectively when given high affinity antibody than when given low affinity antibody. In one experiment, amounts of antibody of high or low affinity which bound 30% of the antigen present into an ammonium sulphate precipitable complex was passively transferred. This must represent more low affinity antibody than high affinity antibody, but even at these doses, animals receiving the higher affinity antibody eliminated the antigen faster than did the animals receiving the low affinity antibody. Experiments in which globulin preparations were transferred showed that the strain differences did not depend on differing properties of the whole serum.

More of the antigen remaining in the serum was complexed to globulin during the first hour after injection in mice receiving high affinity antibody than in those receiving low affinity antibody. Considerable elimination of antigen by high affinity antibody takes place during this time and thus in absolute terms the amount of antigen bound to high affinity antibody is much greater. The need for further study of the size and properties of complexes formed with both high and low affinity antibody is indicated from these preliminary observations.

Though our experiments show that most of the difference between strains depends on their antibody response, the observation that immunized Simpson mice (a strain which is good at active immune elimination) eliminate more antigen when given low affinity antibody than do CBA mice (a strain which is relatively poor at elimination) suggest that some other interstrain differences are also relevant. One of the theories we have suggested (Soothill & Steward, 1971) to explain the failure of some strains of mice to produce high affinity antibody is a defect of macrophage function. Since macrophages are important in the elimination of complexes, it is possible that the same primary abnormality could produce both effects.

It is clear that both quantity and quality of the antibody is important in the immune elimination of fixed amounts of antigen. An infection leading to release of very large amounts of antigen could result in a period of limited antigen elimination even in an animal capable of producing high affinity antibody, because, for a while the animal was in such antigen excess that only antigen excess soluble complex would be formed; this may lead to acute serum sickness. In a low affinity antibody producing animal, soluble complexes may well be formed at any relative concentration of antigen to antibody, and these might aggregate after deposition with consequent phlogogenic activity in the tissues (Lightfoot, Drusin & Christian, 1971). Alternatively, as a result of the failure to eliminate antigen, any complexes formed by the small proportion of high affinity antibody produced would be in antigen excess and they would give rise to the immunopathological lesions. The work of Oldstone & Dixon (1969) gives an indication that this may be so, since strains of mice which have chronic soluble complex disease as a result of LCM virus infection have far more virus in the circulation and tissues than those strains of mice which do not have the nephritis, even though all received the same infecting dose of virus. We studied four of the strains they used, and the animals they showed to be prone to such disease were indeed those poor at immune elimination of the antigen we used.

We have previously reported that animals which produce low affinity antibody when immunized with antigen in saline, produce high affinity antibody when immunized with antigen in Freund's complete adjuvant (Soothill & Steward, 1971). Adjuvant immunization of strains poor at immune elimination, when immunized in saline, makes them effective at immune elimination. This could be of therapeutic significance in chronic soluble complex disease.

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