THE EFFECT OF PASSIVELY ADMINISTERED ANTIBODY ON ANTIBODY SYNTHESIS*, ‡

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It has long been recognized that preexisting antibody, either endogenously formed, or passively administered near the time of the injection of the corresponding antigen, can affect the subsequent antibody response. An excess or, in some cases, even an equivalent amount of passively administered antibody can partially or completely inhibit the primary response in a previously unchallenged animal (1–12) and passively administered or previously synthesized antibody may, under certain conditions, diminish the response to a secondary challenge (10, 13–15). On the other hand, with certain antigens, relatively small amounts of passively administered antibody actually may enhance the antibody response (12, 16–18).

The mechanism by which preexisting or passively transferred antibody suppresses the antibody response is not entirely clear. It could be either by covering the immunogenic sites of the antigen and thereby preventing or diminishing the immunogenic stimulus, or by a direct suppressive action on the cells destined to respond to the antigen. In the broad sense, either of these actions might be considered a "feedback mechanism" since the product of the response, antibody, would serve, directly or indirectly, to prevent or diminish the response. Most of the available observations of this phenomenon are based on experiments which utilize complex antigens and at least equivalent or, more often, large excesses of antibody in relation to the immunogenic challenge and are, therefore, of little help in choosing between these two possibilities (8–14). Those experiments designed to eliminate the excess antibody by exposing potentially immunocompetent cells to passive antibody prior to or during transfer to unresponsive hosts have led to conflicting results (10, 11).

The present studies, in which giant keyhole limpet hemocyanin (KLH) was used as an antigen in rabbits, were undertaken in an attempt to learn more of the mechanism by which antibody affects antibody synthesis. The KLH

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rabbit system has advantages for such a study since the primary antibody response is slowly mounted and well sustained, allowing easy manipulation and observation of its various temporal stages. Also the interaction of KLH and anti-KLH can be quantitated and characterized by immunochemical procedures which afford some insight into the molecular interactions involved in the suppression of the antibody response. The importance of the temporal relationship between administration of antigen and antibody in achieving immunosuppression, the quantitative relationship between the antigenic stimulus and the amount of passively administered antibody needed to effect suppression of the antibody response, and some of the qualitative characteristics of immunosuppressive antibody have been explored. The observations reported strongly suggest that the inhibitory effect of antibody is via neutralization of the antigenic stimulus. Further, they indicate that this neutralization takes place at the level of individual antigenic determinants, not entire antigenic molecules, and that the neutralized material probably exists within or fixed at the surface of cells. Since early primary antibody has very little immunosuppressive effect, it seems questionable whether antibody formed in the primary response is an important regulator of the process of antibody synthesis. Finally, it appears that a very small part of the intravenously administered KLH ever becomes immunogenic, i.e., effective in stimulating antibody formation. In this paper the term immunogen will be used to indicate those antigen molecules or antigen fragments that actually stimulate antibody synthesis, in contrast to the term antigen which indicates all foreign, potentially immunogenic material administered to a subject.

Materials and Methods

Albino male rabbits weighing 5–6 lb. were used as experimental subjects. Associated KLH (mol wt 7.5×10^6) purified by ultracentrifugation (19) was used as antigen in all experiments. Incomplete Freund adjuvant containing KLH used in some immunizations was prepared by mixing in a Waring Blendor 9 parts of Bayol F, 1 part Arlacel C and 10 parts of 0.15 M saline containing the KLH. In some experiments the KLH or purified anti-KLH incorporated in these adjuvants was labeled with ^{131}I (20) and its rates of loss from the injection depot and/or whole body were determined as previously described (21).

Anti-KLH determinations were made with a modified quantitative precipitin technique using dissociated KLH (mol wt 814,000) as previously described (21). These results are expressed as micrograms of dissociated KLH nitrogen precipitated by 1 ml of antiserum at the point where 80% of the KLH added is precipitated (P^{80} units). Under these conditions the anti-KLH–KLH ratio is slightly greater than two (19). Direct determination of the amount of antibody per P^{80} unit by precipitation of labeled antibody from ^{131}I-labeled γ-globulin preparations of known P^{80} from several hyperimmune antisera in the presence of excess KLH gave an average value of 11.0 μg antibody protein/P^{80} unit.

In most studies, the antibody that was passively administered was from two pools of hyperimmune sera from rabbits repeatedly immunized by a variety of routes over a period of 3 or more months. These pools had antibody concentrations of 900 and 300 P^{80} units/ml

respectively. Antibodies from these two pools as measured by the P^{80} technique were equally immunosuppressive. In particular procedures, early primary rabbit anti-KLH, mink or rat hyperimmune anti-KLH, the 7S γ-globulin from the primary or hyperimmune rabbit anti-KLH, and slow and fast 7S antibodies from hyperimmune rabbit anti-KLH or hyperimmune

TABLE I

The Relationship of Time of Administration and Suppressive Effect of Intravenously Transferred Antibody

Passive antibody	No. of rabbits	Time of administration pre (−) or post (+) antigen	Average antibody response	
2 mg KLH i.v.			Day 20	Day 30
P^{80} units			*P^{80} units*	*P^{80} units*
0	63		9.2	5.9
9	4	−1 day	2.5	2.4
9	3	+1 hr	6.5	5.8
9	37	+1 day	0.9	1.6
9	5	+4 day	2.0	2.1
45	4	−1 day	0.3	0.3
45	3	+1 hr	0.8	1.7
45	4	+1 day	0.1	—
45	4	+4 day	2.6	—
90	4	+1 hr	0.3	0.2
90	3	+4 day	0.9	1.4
90	5	+8 day	5.0	4.2
1800	4	+6 day*	1.4	1.7
1800	4	+8 day‡	2.2	2.0
10 mg KLH i.v.				
P^{80} units				
0	6		9.0	6.3
18	4	−1 day	9.2	—
18	4	+1 day	2.4	3.3
100 mg KLH i.v.				
P^{80} units				
0	5		11.5	8.1
90	5	−1 day	20.7	—
90	5	+1 day	12.7	12.3

* Rabbits' own P^{80} 1.1 before passive antibody.

‡ Rabbits' own P^{80} 1.3 before passive antibody.

rabbit anti-abalone hemocyanin (AH) which cross-reacts with KLH were passively administered. These preparations are described in more detail in connection with the experiments in which they were used.

EXPERIMENTAL DESIGN

(*a*) The first experiments were designed to determine the inhibitory effect on the primary antibody response of hyperimmune rabbit antibody passively administered intravenously to rabbits at different times in relation to the intravenous injection of antigen (Table I). With an intravenous challenge of 2 mg KLH, four doses of passively administered antibody were employed (1800, 90, 45, and 9 P^{80} units) and they were administered 1 day before and 1 hr, 1 day, 4 days, 6 days, and 8 days after the antigen. With 10 mg KLH, 18 P^{80} units were given 1 day before and 1 day after antigen, and with 100 mg KLH, 90 P^{80} units were given 1 day before and 1 day after antigen. Antibody levels were determined 20 and 30 days after antigen injection, the time of maximum serum antibody response (21).

TABLE II

The Relationship between Size of Intravenous Antigenic Stimulus and Amount of Intravenously Transferred Immunosuppressive Antibody Given 1 Day Later

KLH	Passive antibody	No. of rabbits	Antibody response	
			Day 20	Day 30
mg	*P^{80} units*		*P^{80} units*	*P^{80} units*
0.2	0	5	3.6	4.0
0.6	0	11	9.9	6.6
0.6	4	10	1.8	2.2
2	0	63	9.2	5.9
2	1	5	10.1	6.5
2	4	11	5.0	4.9
2	9	37	0.9	1.6
2	18	4	0.4	0.8
2	45	4	0.1	—
10	0	6	9.0	6.3
10	27	5	4.1	5.3
10	45	5	1.4	3.0
100	0	5	11.5	8.1
100	90	5	12.7	12.3

(*b*) The second series of observations dealt with the quantitative relationship between the intravenous antigenic stimulus and the passively administered immunosuppressive antibody. A series of intravenous antigenic stimuli (0.6, 2, 10, and 100 mg KLH) was inhibited by a variety of doses of hyperimmune anti-KLH given intravenously 1 day after injection of KLH as shown in Table II. Again, serum antibody levels of the recipients were determined 20 and 30 days after injection of antigen.

(*c*) The third series of experiments was intended to reveal some of the characteristics of the passively administered antisera which were related to their immunosuppressive action. The possible role of nonantibody factors in causing inhibition was checked by attempting inhibition with unrelated hyperimmune antisera. Five rabbits were given 2 mg KLH intravenously followed by 600 P^{80} units of rabbit anti-bovine serum albumin 1 day later. Also, any effect of nonantibody factors in the immunosuppressive anti-KLH sera was looked for by attempting to inhibit the response of rabbits given 2 mg KLH intravenously with serum injected 1 day later which originally contained 900 anti-KLH P^{80} units but had been absorbed at equivalence with KLH before injection.

TABLE III

Effect of Early and Late Intravenously Transferred Antibody on Primary Response to 2 mg KLH

Antibody administered 1 day after KLH.

Type of passive antibody	Passive antibody	No. of rabbits	% of day 20 control response
	P^{80} units		
7 Day adjuvant primary pool	20	4	70
" "	150	4	84
" " 7SγG*	100	4	73
9 Day " "	100	10	60
20 Day normal primary pool	20	4	57
20 Day X-ray primary pool	20	5	67
21 Day adjuvant primary (‡2-47)	20	5	62
48 Day X-ray primary pool	20	4	28
50 Day adjuvant primary (‡2-47)	20	4	25
90 Day adjuvant primary (‡2-47)	20	4	13
" " (‡2-46)	20	4	19
" " (‡2-45)	20	4	32
7 Day normal secondary (‡4-89)§	20	5	8
21 Day " " (‡4-89)§	20	4	5
21 Day " " (‡4-91)§	20	4	16
Hyperimmune pool	9	9	18
Hyperimmune pool plus 9 Day primary antibody	9 + 100	6	14

* 7Sγ-globulin obtained by chromatography on DEAE (23).

‡ Individual rabbit numbers.

§ Second injection of KLH 95 days after first injection.

Next, the relative inhibitory power of rabbit anti-KLH formed at different times after initial immunization was determined (Table III). In order to obtain sufficient amounts of donor primary antibody, either KLH was given in incomplete adjuvant (21) (3 mg KLH in 2 ml adjuvant/rabbit) or the primary response to 2 mg intravenously was enhanced by

X-ray (22) (500 r given 2 hr after intravenous KLH). These procedures increased and accelerated the primary antibody responses. Antibody obtained from rabbits 7 and 21 days after the second of two injections of 2 mg KLH given intravenously 95 days apart was also tested for inhibitory action.

The inhibitory power of anti-KLH obtained from mink and rats repeatedly immunized over a period of more than 3 months was tested in rabbits given 2 mg KLH intravenously. The mink antiserum contained 230 P^{80} units/ml and the rat antiserum 200 P^{80} units/ml. Because the half-lives of rat and mink γ-globulins in rabbits were 1.7 and 1.3 days, respectively, in contrast to approximately 6 days for rabbit γ-globulin, these heterologous antisera were given repeatedly to two groups of rabbits, 1 hr (60 P^{80} units), 2 days (50 P^{80} units), and 5 days (50 P^{80} units) after the injection of KLH in order to maintain their level in the circu-

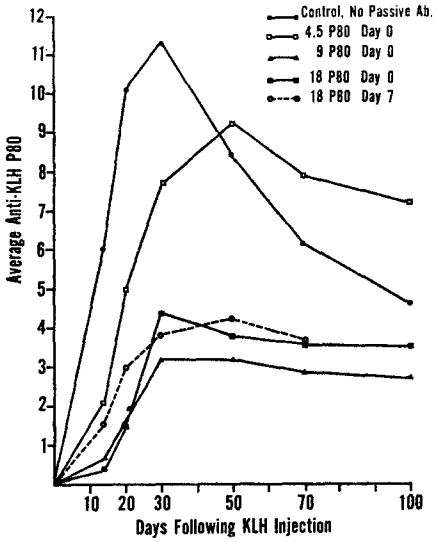

FIG. 1. The immunosuppressive effect of intravenously administered passive anti-KLH on the response to 2 μg KLH in adjuvant.

lation. One group of five rabbits received a single injection of 20 P^{80} units of rat anti-KLH 1 day after 2 mg KLH, and one group of four rabbits received two injections of 50 P^{80} units of mink anti-KLH 1 hr and 2 days after 2 mg KLH.

The immunosuppressive quality of an antibody cross-reactive with KLH was tested. Sera from rabbits hyperimmunized with AH cross-reacted approximately 50% with KLH, i.e., such antisera when tested with KLH had P^{80} values of approximately one-half that when tested with AH. Conversely, hyperimmune anti-KLH precipitated approximately 20% as much AH as KLH and from 20–40% of the anti-KLH antibodies from such sera could be absorbed out with an excess of AH. Three groups of five rabbits were given 2 mg KLH intravenously and 1 day later each received one of three doses of rabbit hyperimmune anti-AH serum. These doses of anti-AH were measured in terms of antibody cross-reacting with KLH, i.e., by the precipitation of KLH, and were 9, 20, and 100 P^{80} units. The anti-KLH response was determined 20 and 30 days after the injection of KLH. 60 days after the initial injection of KLH, these rabbits received a second injection of 2 mg of KLH and the cross-reactivity

of the antibody formed in the secondary response was tested against AH in the P^{80} procedure and compared with the antibody made in rabbits comparably stimulated but without receiving anti-AH.

The immunosuppression produced by slow and fast 7S hyperimmune anti-KLH antibodies as separated by DEAE chromatography was observed. Ammonium sulfate precipitated γ-globulin from the antisera were fractionated by DEAE chromatography according to the

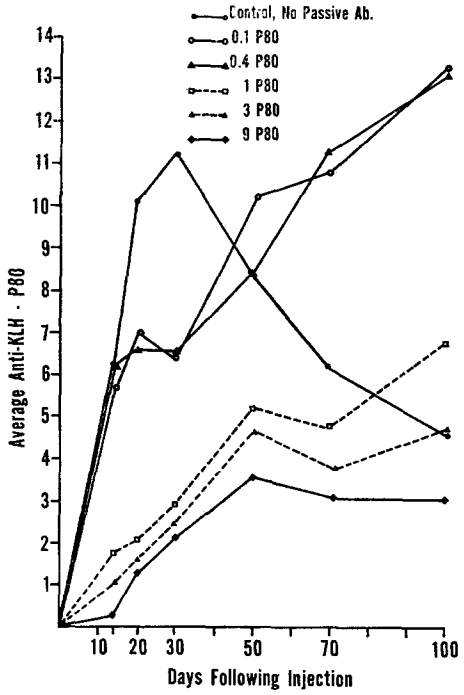

FIG. 2. The immunosuppressive effect of various amounts of anti-KLH incorporated with 2 μg KLH in adjuvant.

method of Sober et al. (23). One group of five rabbits received 9 P^{80} units of slow 7S antibody 1 day after 2 mg KLH and another group of five received 9 P^{80} units of fast 7S antibody. The responses were measured 20 and 30 days after injection of KLH.

(*d*) The fourth set of experiments involved the immunization of rabbits with KLH in incomplete Freund adjuvant and the inhibition of the antibody response with intravenously administered hyperimmune rabbit anti-KLH. As previously reported, 2 μg KLH in 2 ml adjuvant elicits an antibody response slightly greater and better sustained than that induced by 0.6–2 mg KLH given intravenously (21). Five groups of rabbits received 2 μg KLH in a total of 2 ml adjuvant in the four footpads and multiple subcutaneous sites. Simultaneously, three of these groups consisting of 11, 13, and 12 rabbits received, respectively, 4.5, 9, or 18 P^{80} units of anti-KLH intravenously. One group of five rabbits received 18 P^{80} units 7 days after antigen administration and the final group of 39 rabbits served as an antigen only control. Antibody levels in all rabbits were determined 14, 20, 30, 50, 70, and 100 days after antigen injection and are presented in Fig. 1. The rate of elimination of 2μg of ^{131}I KLH in

adjuvant from the adjuvant depot and/or whole body was observed in eight rabbits in the absence of passive antibody.

(*e*) The final experiment was designed to compare the immunosuppressive efficiency of passive antibody distributed throughout the host versus antibody concentrated in the vicinity of the antigen. It also allowed us to determine the precise amount of antibody needed to neutralize the immunogenicity of a known amount of KLH, i.e., the minimum antibody-antigen ratio of a nonimmunogenic mixture. Various amounts of anti-KLH were mixed with KLH and incubated at 37°C for 30 min and at 4°C for 2 days before being incorporated into incomplete adjuvant for injection. 7 rabbits received 2 μg KLH plus 0.1 P^{80}, 1.1 μg anti-KLH in adjuvant; 10 received 2 μg KLH plus 0.4 P^{80}, 4.4 μg anti-KLH; 8 received 2 μg KLH plus 1 P^{80}, 11 μg anti-KLH; 8 received 2 μg KLH plus 3 P^{80}, 33 μg anti-KLH; and 11 received 2 μg KLH plus 9 P^{80}, 99 μg anti-KLH. Levels of anti-KLH in the sera of these recipients were determined 14, 20, 30, 50, 70, and 100 days later and are presented in Fig. 2. The rate of loss of antibody from the adjuvant and the whole animal was determined using ^{131}I-labeled 7S γ-globulin from hyperimmune anti-KLH sera which was 60% precipitable with KLH. The labeled globulin was incorporated into adjuvant alone or with KLH at a ratio of 50:1 and its rate of loss from injection site and whole body determined.

RESULTS

The results of the studies in which immunosuppressive doses of antibody were intravenously administered at various times relative to the injection of antigen are given in Table I. Clearly, 9 P^{80} units of antibody are most effective in suppressing the response to 2 mg KLH intravenously if given 1 day after KLH. The same amount of anti-KLH given 1 day before, 1 hr after or 4 days after KLH was less effective. 45 P^{80} units of anti-KLH was effective in suppressing the response to 2 mg if given 1 day before or 1 hr after KLH but was less effective if given 4 days after. 90 P^{80} units was an effective immunosuppressant if given either 1 hr or 4 days after KLH but was less than 50% suppressive if given 8 days after. 1800 P^{80} units given 6 or 8 days after KLH when the recipients already had anti-KLH levels of their own of 1.1 and 1.3 P^{80} units, respectively, resulted in an apparent 70–80% suppression of antibody response. Actually with 1800 P^{80} units, a significant part of the antibody measured on day 20, and to a lesser extent on day 30, was probably passively administered antibody so that suppression of antibody synthesis may have been greater than the antibody levels indicate. With a challenge of 10 mg of KLH, 18 P^{80} units given 1 day before KLH caused no suppression while the same amount given 1 day after KLH reduced the 20 day response to one-fourth the control level. With 100 mg KLH, 90 P^{80} units given 1 day after KLH produced no immunosuppression and if given 1 day before, KLH may have actually enhanced the antibody response.

The quantitative relationships between intravenous challenges of KLH and immunosuppressive doses of anti-KLH given 1 day later are shown in Table II. First, it is apparent that challenges of 0.6–100 mg of KLH unaccompanied by passively administered antibody give comparable antibody responses 20 and 30 days later while 0.2 mg elicits a significantly smaller response. 4 P^{80} units

causes an 80% suppression of the 20 day response to 0.6 mg KLH and an approximately 50% suppression with 2 mg KLH. 9 P^{80} units causes a 90% suppression of the 20 day response to 2 mg KLH while 18 P^{80} units causes a 95% inhibition and 45 P^{80} units virtually abolishes the 20 day response to 2 mg KLH. When the KLH challenge is raised 5 times, to 10 mg, approximately 5 times larger amounts of anti-KLH are needed to give the same degree of immunosuppression seen with 2 mg KLH. 27 P^{80} units of anti-KLH given with 10 mg KLH gives a slightly greater degree of immunosuppression than 4.0 P^{80} units gives with 2 mg KLH, and 45 P^{80} units inhibits the 20 day response to 10 mg to nearly the same degree as 9 P^{80} units inhibits the response to 2 mg. After 100 mg KLH, 90 P^{80} units had no immunosuppressive effect and much larger amounts of antibody could not be used because they induced anaphylactic reactions.

In the studies dealing with the nature of the inhibitory antibodies, the results were as follows:

Neither hyperimmune anti-bovine serum albumin serum, nor hyperimmune anti-KLH serum previously absorbed with an equivalent amount of KLH had any immunosuppressive effect in the amounts used.

The inhibitory properties of anti-KLH antibodies obtained at different times after immunization of the donor are shown in Table III. Antisera taken 7 and 9 days after adjuvant or X-ray enhanced primary KLH immunization are, at best, weakly immunosuppressive, even in large doses. This is true for both the entire serum or the 7S γ-globulin fraction thereof. Early antibody will not, however, interfere with the immunosuppressive action of hyperimmune antibodies as indicated by the full suppression demonstrated by 9 P^{80} units of hyperimmune anti-KLH in the presence of 100 units of 9 day primary antibody. Anti-KLH antibodies obtained at the 20th or 21st day of normal or enhanced primary responses were mildly immunosuppressive; 20 P^{80} units produced about a 40% inhibition. 20 P^{80} units of primary antisera obtained 48–50 days after immunization produced about a 75% inhibition of the day 20 response. 90 day primary antisera in doses of 20 P^{80} units caused an average of 80% suppression. If such comparisons are valid, the 90 day primary antibodies would appear to be less than one-half as immunosuppressive as hyperimmune antibodies. For donor rabbit 2-47, bled 21, 50, and 90 days after primary immunization, the immunosuppressive properties of its antibodies appeared to increase with time. The immunosuppressive properties of antibodies obtained 7 and 21 days after a secondary challenge appeared to approach those of hyperimmune antibodies.

Mink and rat hyperimmune anti-KLH had a moderate immunosuppressive effect in rabbits. Those rabbits receiving 50 P^{80} units of mink anti-KLH 1 hr and 2 days after 2 mg KLH had average 20- and 30-day responses of 3.8 P^{80} and 3.6 P^{80} respectively and those receiving three injections of mink anti-KLH

totaling 160 P^{80} units had average 20- and 30-day responses of 2.1 P^{80}. The rabbits receiving one injection of 20 P^{80} units of rat anti-KLH given 1 day after KLH had 20- and 30-day P^{80} values of 4.7 and those receiving three injections of rat anti-KLH totaling 160 P^{80} had 20- and 30-day responses of 1.9 P^{80} units. Comparison of the immunosuppressive effects of these antisera with rabbit antisera is difficult because of their more rapid rate of catabolism; it is clear, however, that they are less suppressive.

Hyperimmune anti-AH, which cross-reacts with KLH, was partially immunosuppressive. 9 P^{80} units of anti-AH given 1 day after 2 mg KLH resulted in an average 20 day response of 4.9 P^{80} units, whereas 20 P^{80} units given similarly resulted in average 20-day responses of 2.6 P^{80}, and 100 P^{80} units an average 20 day response of 2.5. At the 9 P^{80} dose, anti-AH appeared about 50% as effective in suppressing the anti-KLH response as hyperimmune anti-KLH. However, even at a dose of 100 P^{80} units, the anti-AH was not as strongly immunosuppressive as 9 P^{80} units of anti-KLH. The secondary anti-KLH made in the rabbits with primary anti-KLH responses partially suppressed with anti-AH did not cross-react with AH differently than control secondary anti-KLH sera.

Hyperimmune anti-KLH from slow and fast 7S γ-globulin fractions both inhibited similarly and in a manner identical to the same amount of anti-KLH of whole serum.

The response of rabbits to 2 μg KLH in incomplete Freund adjuvant was suppressed by intravenously administered hyperimmune anti-KLH as indicated in Fig. 1. 4.5 P^{80} units given at the time of injection of antigen caused a delay and an approximate 20% suppression of the antibody response while 9 P^{80} units similarly administered caused an 75% inhibition and 18 P^{80} units slightly less. The injection of 18 P^{80} units 7 days after antigen also resulted in a 60–70% inhibition. With the adjuvants prepared in these experiments, ^{131}I-labeled KLH escaped from the depot site and/or the whole rabbit with a half-life of about 17 days during the first 2 postinjection weeks and thereafter with a half-life of about 50 days.

The immunosuppressive effect of anti-KLH incorporated directly with 2 μg KLH in incomplete adjuvant was considerably greater than when the anti-KLH was given intravenously (Fig. 2). 0.1–0.4 P^{80} units, or 1.1–4.4 μg, of anti-KLH caused about a one-third suppression of the 20 and 30 day antibody levels which were followed by increasing antibody levels to control levels or greater at 70–100 days. 1 P^{80}, or 11.0 μg anti-KLH, which presumably saturated the KLH, caused an 80% suppression of 20–30-day levels after which antibody levels reached 50% of control maximum by 50 days. 3 and 9 P^{80} units, 33 and 99 μg anti-KLH caused only a slightly greater suppression than 1 P^{80} unit throughout the 100 day observation period. Using ^{131}I-labeled purified hyperimmune anti-KLH, it was determined that, in extreme antibody

excess, associated KLH could combine with, and was saturated by, approximately 4 times its weight of antibody. A late increase in antibody response after injection of antigen-antibody complexes in adjuvant has been observed previously and attributed to dissociation of the complexes with stimulation by free antigen (8).

^{131}I-labeled anti-KLH incorporated in these adjuvants escaped from the adjuvant depots and disappeared from the whole body of rabbits at a rate with a half-life of 17 days during the first 2 postinjection weeks and thereafter at a half-life of somewhat greater than 30 days.

DISCUSSION

The temporal and quantitative aspects of the immunosuppressive effects of passive antibody, together with the previously observed behavior of intravenously administered KLH in rabbits (21), give some indications of the mechanism of action of the passive antibody. After intravenous administration, KLH is cleared from the circulation rapidly so that within 1 hr $< 10\%$ is circulating and within 6 hr, only 1.5% (21). Clearance of antigenic material from the entire body is impossible to quantitate but based on studies employing ^{131}I-labeled KLH, it appears that after 1 day somewhat $< 10\%$ of the intact KLH may persist, after 2 days $<7\%$, and after 3 days $<3\%$. Thus, the rabbit appears capable of rapidly disposing of the greater part of this antigen by nonimmunologic means. If the rabbit is allowed 1 day after primary injection to eliminate most of the KLH, then the injection of relatively small amounts of anti-KLH will inhibit the antibody response. At this time, injection of only 5–10% of the anti-KLH which would react at equivalence with the injected antigen is strongly immunosuppressive. Anti-KLH administered 1 day before and, more particularly, 1 hr after antigen must encounter most of the injected KLH in the circulation before the host eliminates it and, therefore, does not suppress the immune response efficiently, although very large amounts of antibody given at these times are suppressive. Of the 9 P^{80} units of anti-KLH given 1 day before 2 mg KLH, only that portion which has equilibrated into the extravascular fluids (about 50%) can escape immediate intravascular reaction with the total dose of the injected KLH and it is probably this anti-KLH in the extravascular fluids which later accounts for the observed partial immunosuppression.

That passively administered antibody can interfere with an anti-KLH response already underway is evident by the suppressive effect of as little as 9 P^{80} units given 4 days after 2 mg KLH and of 1800 P^{80} units given 6 and 8 days after 2 mg KLH (Table I). The rabbits injected at 6 and 8 days already had made detectable circulating anti-KLH prior to the administration of passive anti-KLH. Even with KLH administered in adjuvant, passive antibody given 1 wk later exerted a strong suppressive effect. In these instances it would seem likely that the passively administered antibody must be reacting with antigen

in or on responding cells or perhaps in the process of transfer between cells. These results are in agreement with earlier reports of others who used different host-antigen systems (8, 10, 12, 15).

Actually there is no evidence that the presence of anti-KLH prior to the administration of KLH has any immunosuppressive effect such as would be expected if the antibody had a direct effect on potentially responsive cells. If passive antibody directly affected responsive cells, then 9 P^{80} units, which was an effective immunosuppressant 1 day after 2 mg KLH, would presumably be capable of suppressing most of the potentially responsive cells in a rabbit since 2 mg KLH stimulates a near maximum primary response. However, twice this amount of anti-KLH, 18P^{80} units, given 1 day before 10 mg KLH, or 90 P^{80} units given 1 day before 100 mg KLH had no immunosuppressive effect and the latter may even have enhanced the response. Thus, no direct adverse effect of passively administered antibody on potentially responsive cells was observed.

The close parallelism between the size of the antigenic challenge and the amount of passive antibody needed to suppress the subsequent immune response strongly suggests that the passive antibody acts by neutralizing retained antigen. Thus, 4 P^{80} units will suppress the response to 0.6 mg KLH to nearly the same degree that 9 P^{80} units suppresses the response to 2 mg and 45 P^{80} units the response to 10 mg. Similarly, 4.0 P^{80} units suppresses the response to 2 mg KLH to nearly the same extent as 27 P^{80} units does the response to 10 mg KLH. Also, at each dose level of antigen, the immunosuppression achieved appears proportional to the amount of passive antibody given. In terms of absolute amounts, however, the quantity of passive antibody is far less than could react with the total dose of antigen and is probably reacting with the traces of residual antigen not previously eliminated by the host's nonimmune mechanisms.

If one can relate the size of the antibody response to the amount of active immunogenic material present in the animal, i.e., that antigen actually involved in stimulating antibody formation, it seems that a relatively constant amount of immunogen results from challenges with amounts of KLH ranging from 0.6 mg to 100 mg [also 1000 mg (21)] since the associated maximum antibody levels are quite similar. However, since larger amounts of passive antibody are needed to suppress responses to the larger doses of antigen, it would appear that amounts of nonimmunogenic antigen proportional to the total antigen given may be retained in a form capable of combining with the passively administered antibody. Such nonimmunogenic antigen might be inactive because of its anatomical position, or its immunochemical form or it might merely represent an excess of potential immunogen to which the host is incapable of responding.

That the passive antibody acts by neutralizing antigen rather than directly on immunocompetent cells is further suggested by the experiments using KLH

in adjuvant. Antibody incorporated directly with the KLH in the adjuvant was about 10 times as effective in immunosuppression over the first 30 days of the response as was antibody given intravenously (Figs. 1 and 2). While the shapes of the suppressed antibody response curves are not comparable, the 20 and 30 day levels in rabbits receiving 4.5 P^{80} units intravenously and 0.4 P^{80} units in adjuvant are similar as are those of rabbits receiving 9 P^{80} units intravenously and 1 P^{80} in adjuvant.

The immunosuppressive effect of passively administered heterologous antibodies further suggests that antibody acts by neutralizing antigen. A non-antigen receptor on potentially responsive cells for heterologous antibodies would seem unlikely. That the heterologous antibodies were less effective than homologous antibodies in this situation might be expected for at least two reasons. First, the heterologous γ-globulins have much shorter half-lives in the rabbit than do homologous. However, when repeated injections of heterologous anti-KLH were used, an 80% suppression was achieved. Second, species other than the rabbit may make less avid antibody to KLH and may respond to different antigenic determinants on the KLH molecules than do rabbits and, thus, provide less effective suppressive antibody for rabbits than do homologous antibodies. The difference between these observations and those of Uhr and Baumann (8) in which heterologous antibodies were more immunosuppressive than homologous may be related to the routes of administration used. Administration of heterologous antibody in adjuvant (8) would minimize the effect of any difference in circulating half-life between homologous and heterologous antibodies.

It is of interest that when minimal doses of intravenous antigen or antigen in adjuvant were employed to give roughly comparable antibody responses, both responses were suppressable by similar amounts of intravenous passive antibody in spite of the fact that widely different amounts of antigen had been injected. If passively administered antibody exerts its suppressive effect by neutralizing immunogen, it would follow that 2 μg KLH in adjuvant and 2 mg intravenously provide comparable amounts of immunogen. In this case, $<1/1000$ of the intravenously administered KLH would become immunogenic.

Two sets of observations suggest that passive antibody blocks the immunogenic stimulus of KLH at the level of individual antigenic determinants and not at the level of the entire antigenic molecule. The first of these was the failure of rather large amounts of cross-reacting anti-AH to suppress completely the anti-KLH response. As the experiments were designed, the amounts of anti-AH used, and which were only partially immunosuppressive, were capable of reacting with many more KLH molecules than the complete, or nearly complete, immunosuppressive doses of anti-KLH. However, there undoubtedly were many KLH specific determinants to which the anti-AH sera contained no antibodies and which were, therefore, not blocked. The second observations

came from the experiments employing anti-KLH and KLH together in adjuvant. Effective immunosuppression was achieved only when saturating amounts of anti-KLH were incorporated with the KLH. Equivalent amounts of anti-KLH sufficient to precipitate all the KLH but not cover all antigenic determinants were only slightly immunosuppressive. Thus, it seems likely that in immunosuppression the KLH molecule as a whole is not neutralized by passive antibody but that individual determinants may be blocked. These observations differ from those of Uhr and Baumann (8) which showed that three antitoxin molecules could effectively neutralize the immunogenicity of one toxin molecule, an amount of antibody too small to cover all of the antigenic determinants of the toxin molecule.

Finally, the present data raise questions concerning the role of antibody as a regulator of antibody synthesis in the course of normal immune responses. Unfortunately, KLH may be a poor antigen for studies on the normal mechanisms of termination of the antibody response since the primary response to this antigen is well sustained. However, it is clear that antibodies, even 7SγG antibodies, formed in the first weeks of the primary response have very little immunosuppressive power in contrast to antibodies formed after repeated immunizations. It is possible that extremely large amounts of early primary antibody might be suppressive, but certainly no quantitative comparisons between natural events and those seen after passive administration of hyperimmune antibody to animals early in a primary response are warranted. It may be that at any time during normal responses, the antibody forming cells can successfully compete for available immunogen with the antibody normally in the circulation at that time. This would fit with the several observations failing to demonstrate an immunosuppressive effect of hyperimmune antibody on a late or secondary antibody response. In this situation the antibody forming cells may be equal to the task of competing with the passive antibody.

One possible role of antibody on antibody formation may be to increase with time the spectrum of determinants on the antigen to which the host responds. Early in an immune response, antibody is made to a limited number of determinants on the antigen and this number increases with the length of the response, thereby increasing the heterogeniety of the antiserum and no doubt accounting at least in part for its increasing cross-reactivity with other antigens. As relatively large amounts of antibody are formed to the most immunogenic determinants on an antigen, these sites on the immunogenic material in the animal may be partially neutralized and the host then responds to the remaining uncovered sites of lesser initial immunogenicity.

Throughout the present experiments, analyses of the various inhibited responses for mercaptoethanol sensitive 19S (MES) and resistant 7S (MER) antibodies were made. The relative amounts of these antibodies in normal responses have been previously reported and the large amount of MES antibody

synthesized throughout the responses noted (21). In the partially inhibited responses, the relative concentrations of MES and MER antibodies were not significantly different from those seen in uninhibited responses. This indicates that the inhibition induced by hyperimmune, largely MER, antibody affected the synthesis of both MES and MER antibody. These observations fit with those reported earlier (21) indicating that in this system at least, the presence of MER antibody does not particularly suppress MES antibody formation.

SUMMARY

Suppression of the primary response of rabbits to intravenously administered KLH can be achieved with very small amounts of hyperimmune anti-KLH administered a day later since the rabbit apparently rapidly eliminates most of the KLH by nonimmunologic means. The amount of passive anti-KLH needed to achieve immunosuppression was directly proportional to the dose of injected antigen.

Antibody passively administered as much as 6–8 days after antigen still can be strongly immunosuppressive, which suggests that the antibody must be reacting with immunogen in or on responding cells or perhaps in the process of transfer between cells.

There was no evidence that the presence of passively administered hyperimmune anti-KLH prior to the injection of antigen had any immunosuppressive action beyond the direct neutralization of the injected antigen.

When KLH was injected in Freund adjuvant, anti-KLH incorporated with the KLH in the adjuvant was much more efficient in causing immunosuppression than anti-KLH given intravenously.

The primary responses to 2 mg KLH given intravenously and 2 μg given in adjuvant reached approximately equal peaks and were suppressible by comparable amounts of intravenously administered anti-KLH.

Two observations suggest that passive antibody neutralizes the immunogenic stimulus at the level of individual antigenic determinants and not merely by aggregating or precipitating entire antigenic molecules. First, anti-abalone hemocyanin (AH) which cross-reacts approximately 50% with KLH was only partially immunosuppressive even in extremely large amounts, i.e., amounts which could react with and precipitate much more KLH than could the smaller but more suppressive doses of anti-KLH. Second, when KLH and anti-KLH were given together in adjuvant, effective immunosuppression was achieved only with amounts of anti-KLH sufficient to saturate or cover virtually all available antigenic determinants.

The immunosuppressive quality of passive antibody increases with time after immunization and with repeated immunization of the donor. In view of their relatively weak immunosuppressive properties, antibodies formed in the first weeks of a primary response may not contribute significantly to the turning off

of the antibody response. In any event, results obtained by passive transfer of hyperimmune antibody to animals early in a primary response cannot be applied to the natural events in a primary response.

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