

REGULATION OF THE IMMUNE RESPONSE: SUPPRESSIVE AND
ENHANCING EFFECTS OF PASSIVELY ADMINISTERED
ANTIBODY*

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(Received for publication 12 January 1971)

We have previously found that passively administered antiserum can simultaneously suppress and enhance the immune response against different determinants of the same antigen molecule (1). This observation was made while studying the regulation of the immune response of rabbits to guinea pig γ_2 -immunoglobulin; passively administered anti-F(ab')₂ antiserum suppressed the response to the F(ab')₂ region of the antigen molecule, while concomitantly augmenting antibody formation against the Fc region.

In the present studies we have attempted to clarify the mechanisms of these contrasting phenomena. In particular we wanted to determine: (a) Whether the observed intramolecular regulatory effects of passively administered antibody might be peculiar to the antigen and antisera used. The results which we now report, using a second and distinct antigen-antibody system with human secretory immunoglobulin A (IgA) as antigen, are remarkably parallel to the previous findings, thereby supporting the concept that these intramolecular regulatory effects may be of general occurrence in the immune response. (b) Whether the mechanisms of suppression and enhancement are directly inter-related. Although it has not been possible to answer this question unambiguously, our findings support the idea that they are independent phenomena.

Materials and Methods

Proteins.—

Serum IgA: Human IgA myeloma protein Cat (γ_{A1} , K) was isolated from ascitic fluid by starch block electrophoresis (2).

Secretory IgA: Lyophilized pooled human colostrum was restored to the original volume by

* This investigation was supported by National Institutes of Health grants AI-09738, AI-08499, and training grant AI-00392-02.

† This is part of a doctoral thesis to be submitted by C.S.P. to the Graduate School of Arts and Science of New York University, Department of Basic Medical Sciences.

§ Career Scientist of the Health Research Council of the City of New York, Contract I-474.

|| Career Scientist of the Health Research Council of the City of New York, Contract I-558.

the addition of distilled water. The isolation procedure for the secretory IgA was essentially that of Cebra and Robbins (3). The colostrum was centrifuged at 4000 rpm for 10 min, after which the lipid at the surface was removed and the underlying liquid was centrifuged at 30,000 rpm (# 30 rotor) in a Spinco L2-65 ultracentrifuge for 2 hr at 4°C. The milky top portion was removed and the remaining liquid was brought to pH 4.6 by the addition of 1 N acetic acid to precipitate residual casein. The preparation was then dialyzed against 0.01 M sodium phosphate, pH 7.0, and passed with the same buffer through a column of DEAE-cellulose (100–200 ml of packed DEAE-cellulose per 100 ml of colostrum) previously equilibrated with the same buffer. When the optical density of the effluent had returned to the baseline, the above buffer plus 0.1 M NaCl was passed through the column, and the protein subsequently eluted was passed through Sephadex G-200 in 0.15 M NaCl. The void volume contained secretory IgA; it was determined to be pure by immunoelectrophoresis at 10 mg/ml with two different rabbit antisera against whole colostrum, and had an $S_{20, w} = 10.6$ at a concentration of 0.6 mg/ml (ultraviolet optics). The purity of the secretory IgA preparation was also confirmed by the observation that when it was labeled with ^{125}I , it was 100% precipitable by an antiserum specific for the secretory component. From 5–10 mg of pure secretory IgA were obtained per milliliter of original colostrum.

Guinea pig γ_2 -immunoglobulin: Purchased from Pentex, Inc., Kankakee, Ill., and further purified by passage through DEAE-cellulose (4).

Guinea pig $F(ab')_2$ fragments: $F(ab')_2$ fragments of purified guinea pig γ_2 -immunoglobulin were prepared by pepsin digestion (5). Their purity was confirmed by double diffusion in agar gel with a rabbit anti- γ_2 -immunoglobulin antiserum where a reaction of partial identity with intact γ_2 -immunoglobulin molecules was obtained.

Chicken ovalbumin: Crystallized chicken ovalbumin was obtained from Pentex, Inc.

Rabbits.—Randomly bred rabbits, weighing 2–2.5 kg, were obtained from Bristol Rabbit Farms, Nassau, N.Y.

Antisera.—

Antiserum vs. serum IgA: Rabbits were each immunized intramuscularly in several sites with a total of 2 mg of IgA myeloma protein emulsified in complete Freund's adjuvant (Difco Laboratories, Detroit, Mich.). They were bled several times between 4 and 8 wk after immunization and the sera from various bleedings were pooled. By double diffusion in agar gel the antiserum gave a reaction of identity between the IgA myeloma protein and secretory IgA. Hereafter, this antiserum will be referred to as "anti-serum IgA antiserum." Separate pools of such antisera, prepared from different groups of rabbits, were used in each of the two experiments performed.

Antiserum vs. guinea pig $F(ab')_2$ fragments: Anti- $F(ab')_2$ antiserum was obtained from rabbits which had been immunized in the footpads with 0.5 or 1.0 mg of the $F(ab')_2$ fragments of guinea pig γ_2 -immunoglobulin incorporated in complete Freund's adjuvant. By double diffusion in agar gel these antisera gave reactions of identity between $F(ab')_2$ fragments and whole γ_2 -immunoglobulin molecules. A different pool of antisera from different bleedings and/or rabbits was used in each of the three experiments performed.

Purified rabbit γG -immunoglobulin from anti-guinea pig $F(ab')_2$ antiserum: The γG -immunoglobulin of rabbit anti- $F(ab')_2$ antiserum was separated either by direct passage of the serum (dialyzed against 0.02 M sodium phosphate buffer, pH 7.7) through a column of DEAE-cellulose equilibrated with the same buffer, or by passage after initial precipitation at 4°C with one-third saturated $(\text{NH}_4)_2\text{SO}_4$ (6). The eluted γG -immunoglobulin was dialyzed against 0.15 M NaCl and then concentrated by ultrafiltration. In one experiment the concentrated preparation was passed through a column of Sephadex G-200 equilibrated with 0.1 M tris (hydroxymethyl)aminomethane (Tris)-HCl buffer, pH 8.0, containing 0.5 M NaCl, and in this instance no γM -immunoglobulin was observed in the eluate as determined by optical density

at 280 nm. By immunoelectrophoresis at a concentration of 6 mg/ml, with two different sheep antisera against rabbit γ M-immunoglobulin, the γ G preparation contained no γ M-immunoglobulin. Finally, normal rabbit serum was added to give a final concentration of about 10% serum (v/v), and the preparation was stored at -15°C until use.

F(ab')₂ fragments of rabbit γ G-immunoglobulin vs. guinea pig F(ab')₂ fragments: These were prepared by pepsin digestion (5) and purified by passage through a column of Sephadex G-200 equilibrated with 0.1 M Tris-HCl buffer, pH 8.0, containing 0.5 M NaCl. The absence of any intact γ G molecules in the F(ab')₂ preparation was confirmed by double diffusion in agar gel with several sheep and goat antisera specific for the Fab and Fc regions of rabbit γ G-immunoglobulin.

Antiserum vs. complete Freund's adjuvant: Several rabbits were injected with 2 ml of an emulsion of equal volumes of saline and complete Freund's adjuvant (CFA)¹ divided among the four footpads. 6 wk later the animals were bled and their sera were pooled.

Normal rabbit serum (NRS): Obtained from Pel-Freez Biologicals, Inc., Rogers, Ark.

Immunization of Experimental Rabbits.—Rabbits used in the different experiments were immunized with antigens incorporated in CFA. Each animal received 0.5 mg of guinea pig γ ₂-immunoglobulin or human secretory IgA, or 1 mg of ovalbumin, in a 2 ml emulsion distributed among the four footpads.

Administration of Antiserum to Rabbits.—In all experiments antiserum was injected at several intervals into the marginal vein of the ear. Control groups received comparable volumes of normal rabbit serum or irrelevant immune serum.

In the two studies in which secretory IgA was used for active immunization, the anti-serum IgA antiserum was not diluted before injection, and a total of about 60 mg of antibody was administered per rabbit.

In the study of the effect of different doses of anti-guinea pig F(ab')₂ antiserum on the response to guinea pig γ ₂-immunoglobulin, three different dilutions of the anti-F(ab')₂ antiserum pool were prepared with NRS. The original pool (containing 2.6 mg of antibody per milliliter) and the three additional dilutions had successive fivefold differences in their concentrations of anti-F(ab')₂ antibody. Four groups of rabbits were injected intravenously at several intervals with equal volumes of the respective serum dilution, so that each rabbit in a given group received a total of 60, 12, 2.4, or 0.48 mg of anti-F(ab')₂ antibody.

In the remaining two experiments the original preparations of whole anti-guinea pig F(ab')₂ antiserum, the derived γ G-immunoglobulin, or the F(ab')₂ fragments of the γ G-immunoglobulin were diluted to the desired concentration with saline and NRS to yield solutions of comparable total protein content. The effects of 3 or 12 mg of whole antibody molecules, and of 20 mg of antibody F(ab')₂ fragments were explored.

Radioiodination of Proteins.—Purified human secretory IgA, guinea pig γ ₂-immunoglobulin, and the F(ab')₂ fragments of γ ₂-immunoglobulin were iodinated with ¹²⁵I (New England Nuclear Corp., Boston, Mass.) according to the iodine monochloride technique (7).

Antibody Determinations.—Quantitative precipitation tests (8) were performed to determine the antibody content of rabbit sera obtained at various times after immunization. The antisera were mixed with the corresponding radioiodinated antigens, and the amount of antigen precipitated in each tube was calculated from its known specific activity. Immune precipitates were dissolved in 0.02 M sodium dodecyl sulfate and their optical densities were read at 280 nm.

In the experiments in which secretory IgA was used as antigen, the total amount of anti-secretory IgA antibody present was directly measured by quantitative precipitation with purified secretory IgA. The amount of antibody produced against the secretory component

¹ *Abbreviations used in this paper:* CFA, complete Freund's adjuvant; NRS, normal rabbit serum.

portion of the molecule was determined as follows: First, the antisera were absorbed with normal human serum (three volumes normal human serum:five volumes antiserum) to remove antibodies against α and L chains. (The effectiveness of this absorption was confirmed by the absence of a precipitation line between the absorbed sera and normal human serum during double diffusion in agar gel.) The amount of antibody against secretory IgA which remained after absorption (i.e., antibody specific for the secretory component) was determined by quantitative precipitation with secretory IgA.² Finally, the content of anti-serum IgA antibody in each sample was considered to be the difference between the measured amounts of anti-secretory IgA antibody and anti-secretory component antibody.

In the experiments in which guinea pig γ_2 -immunoglobulin was used as antigen, the amounts of anti- γ_2 -immunoglobulin and anti-F(ab')₂ fragment antibodies in each serum were measured by quantitative precipitation with the corresponding radiiodinated antigens. The amount of anti-Fc antibody in each sample was considered to be the difference between the two.

The antibody response of rabbits immunized with ovalbumin was determined by quantitative precipitation with unlabeled ovalbumin.

RESULTS

Augmentation of the Immune Response to the Secretory Component of Secretory IgA by the Passive Administration of Antiserum against Serum IgA

The secretory IgA molecule contains distinct immunologically and chemically defined regions, α and L chains like those in serum IgA, plus an additional moiety, the secretory component, which is not present in serum IgA or any other serum protein (11). In our experience, when secretory IgA is used as an antigen, most of the precipitating antibody elicited is directed against the region comparable to serum IgA, i.e. α and L chains, rather than against the secretory component. In fact we have been unable to induce good precipitating antisera against the secretory component of secretory IgA with conventional immunization schemes (see also reference 12). For these reasons, secretory IgA appeared to represent an analogous yet independent system with which we might confirm and extend our previous findings with guinea pig γ_2 -immunoglobulin. In the present studies we attempted to enhance the immune response against the weakly immunogenic determinants of the secretory component by administering antiserum specific for serum IgA to rabbits immunized with secretory IgA. The results of two such experiments are presented in Table I.

Two principal points which emerge from the data are: (a) In both studies the production of antibody specific for the secretory component is significantly enhanced (more than twice as great) in the experimental rabbits receiving anti-serum IgA antibody in comparison with the control rabbits. (b) The enhancing and suppressive effects of passively administered antibody are not parallel in time; augmentation becomes evident later than does suppression.

² Normal human serum has recently been reported to contain from 5 (9) to 35 (10) $\mu\text{g/ml}$ of secretory IgA. Thus, small amounts of anti-secretory component antibody may be removed during the absorption procedure. However, the levels of anti-secretory component antibody measured would not be affected to a significant extent.

Thus, by 16 days suppression of the response toward determinants in common with serum IgA is already marked, but there is not yet an increase in the level of anti-secretory component antibody. The same effect is also evident in the experiments below.

TABLE I
Antibody Produced after Active Immunization with Secretory IgA by Rabbits Receiving Anti-Serum IgA Antiserum Passively

Exp. No.	Days after immunization	Rabbits*	Mean antibody levels (OD at 280 nm/ml antiserum)†	
			Anti-serum IgA (\pm SEM)	Anti-secretory component (\pm SEM)
1	16	Experimental	0.54	0.49
		Control	2.50	0.54
	23	Experimental	1.15	1.73
		Control	4.63	0.70
2	29	Experimental	3.53 \pm 0.93	1.31 \pm 0.28§
		Control	4.17 \pm 0.49	0.49 \pm 0.03§
	35	Experimental	3.22	1.29
		Control	4.73	0.53

* A different pool of antiserum was used in each experiment. In experiment 1, five experimental rabbits each received a total of 57 mg of anti-serum IgA antibody distributed in the following proportions: 28% (on the day preceding active immunization), 28% (day 1 after immunization), 19% (days 3 and 7), and 6% (day 10). In experiment 2, four experimental rabbits each received a total of 60 mg of anti-serum IgA antibody distributed in the following proportions: 25% (day preceding immunization), 25% (day 1 after immunization), 17% (days 4, 8, and 20). Control rabbits (five in each experiment) received comparable volumes of NRS.

† Where SEM is not given in the table, this indicates that titrations were performed on pools obtained by mixing equal volumes of serum from the animals in each group. In our experience, such determinations approximate very closely the arithmetic means determined by titration of the individual sera from each group.

§ The difference between these means is statistically significant at the 1% level (Student's *t* test).

Augmentation of the Immune Response to the Fc Region of Guinea Pig γ_2 -Immunoglobulin by Passive Anti-F(ab')₂ Antiserum

Relationship between the Suppressive and Enhancing Effects of Antiserum.— In order to determine whether the augmentation of the immune response to certain determinants on a molecule is dependent upon the suppression of antibody formation against other determinants on the same molecule, we administered varying doses of rabbit anti-F(ab')₂ antiserum to rabbits actively immunized with whole guinea pig γ_2 -immunoglobulin, and observed whether

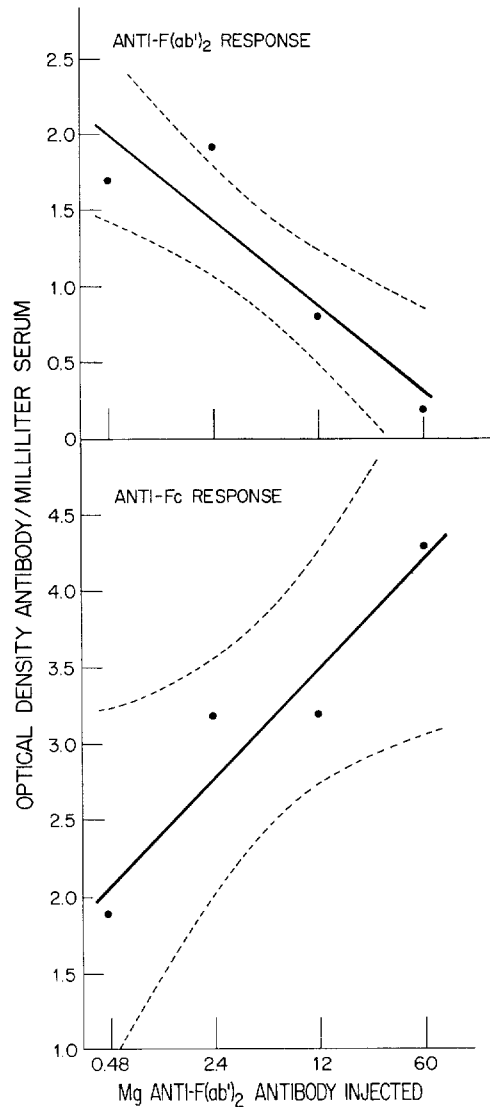


FIG. 1. Regression lines relating the amounts of anti-F(ab')₂ and anti-Fc antibody produced to the dose of passively administered anti-F(ab')₂ antibody (semilogarithmic plot).

Antibody content (arithmetic means) at day 35 after immunization was determined by titration of sera of individual rabbits; these group means are indicated by solid dots. The 95% confidence interval for each regression line is delineated by dotted lines.

Mean antibody levels (OD/ml serum) in the control group receiving normal rabbit serum were 1.2 (anti-F(ab')₂) and 1.5 (anti-Fc). Anti-Fc antibody production by groups receiving 60, 12, or 2.4 mg of antibody was significantly enhanced in relation to the control group ($P < 0.01$, < 0.05 , < 0.05 , respectively; Student's t test). Anti-F(ab')₂ antibody synthesis by groups receiving 60 or 12 mg of antibody was significantly suppressed in relation to the control group ($P < 0.001$; $0.05 < P < 0.10$, respectively). For experimental details see legend to Fig. 2.

enhancement of the immune response to the Fc region could be obtained with doses of antibody which did not cause discernible suppression of antibody synthesis against the homologous region (Figs. 1 and 2).

The antibody content of the sera of individual rabbits at 35 days after immunization was used to plot regression lines relating the amounts of anti-F(ab')₂ and anti-Fc antibody produced to the logarithm of the dose of anti-F(ab')₂ antibody administered (Fig. 1). Analysis of variance indicates that the linear regression obtained with regard to the amount of anti-F(ab')₂ antibody produced by the different groups is statistically significant ($P < 0.01$), as is the

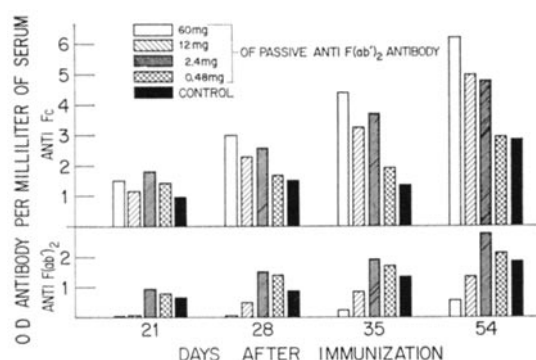


FIG. 2. Antibody produced after active immunization with guinea pig γ_2 -immunoglobulin by rabbits receiving different amounts of passive anti-F(ab')₂ antibody.

Each group (of six rabbits) received the following volumes of its respective dilution of anti-F(ab')₂ antiserum per animal (see Materials and Methods): 7 ml (1 day preceding active immunization), 7 ml (day 3 after immunization), 7 ml (day 7), and 2 ml (day 12). A control group (of eight rabbits) received normal rabbit serum instead. Titrations were performed on pools obtained by mixing equal volumes of serum from the animals in each group.

linear regression obtained for the amount of anti-Fc antibody synthesized ($P < 0.05$). In both cases, the deviations from regression are not significant. Thus, there is a relation between the amount of anti-F(ab')₂ antibody passively administered and the degree of (a) suppression of anti-F(ab')₂ antibody synthesis and (b) enhancement of anti-Fc antibody synthesis.

However, evaluation of these data in relation to mean antibody production by control rabbits receiving normal rabbit serum (see legend, Fig. 1; also Fig. 2) indicates that the enhancement observed is not directly dependent upon the suppression of the response to determinants of the F(ab')₂ region. The higher doses of passive antibody (60, 12 mg) caused suppression of anti-F(ab')₂ antibody production and also enhancement of anti-Fc antibody formation. A smaller dose (2.4 mg) of passive anti-F(ab')₂ antibody could also increase anti-Fc antibody formation. For example, at day 35 the enhancement caused

by 2.4, but not 0.48 mg, was significant at the 5% level (Student's *t* test). Interestingly, however, the 2.4 mg dose did not suppress anti-F(ab')₂ antibody production but instead appeared to augment it as well as anti-Fc antibody

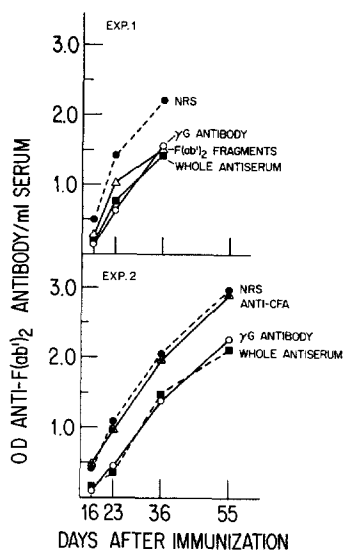


FIG. 3. Anti-F(ab')₂ antibody produced after active immunization with guinea pig γ_2 -immunoglobulin by rabbits receiving passive anti-F(ab')₂ antibody in different forms.

In experiment 1 (upper half) two groups of rabbits received a total of 3 mg of antibody per animal in either whole anti-F(ab')₂ antiserum or purified γ G-immunoglobulin, distributed in the following proportions: 30% (1 day preceding active immunization), 30% (day 3 after immunization), 30% (day 7), and 10% (day 12). The control group received comparable volumes of NRS. Rabbits receiving rabbit F(ab')₂ fragments specific for guinea pig F(ab')₂ were each injected with a total of 20 mg of antibody fragments administered by eight daily injections beginning on the day of immunization. All groups consisted of five rabbits.

In experiment 2 (lower half) two groups of rabbits received a total of 12 mg of antibody per animal in either whole anti-F(ab')₂ antiserum or purified γ G-immunoglobulin, administered as in experiment 1. The rabbits receiving anti-CFA antiserum or NRS were injected with comparable volumes. The groups contained the following numbers of rabbits: whole antiserum, five; γ G antibody, five; anti-CFA, five; NRS, seven.

In all cases titrations were performed on pools obtained by mixing equal volumes of serum from the animals in each group.

formation. Because of the great variation in the amount of antibody produced by individual rabbits, this apparent increase in anti-F(ab')₂ antibody production was not statistically significant at the 5% level (for the group receiving 2.4 mg, $0.05 < P < 0.10$, at day 35).

Nonetheless, an important point which emerges from the group receiving 2.4 mg is that, in contrast to the groups receiving higher doses, significant

enhancement of the anti-Fc antibody response can occur without concomitant suppression of anti-F(ab')₂ antibody synthesis. Furthermore, the levels of anti-F(ab')₂ and anti-Fc antibody at different times after immunization (Fig. 2) indicate that the suppressive effect occurs early in the immune response, whereas enhancement is evident only at a later time.

Ability of γ G Antibody or Fragments Specific for Guinea Pig F(ab')₂ to Enhance the Anti-Fc Response.—In view of contradictory reports attributing the

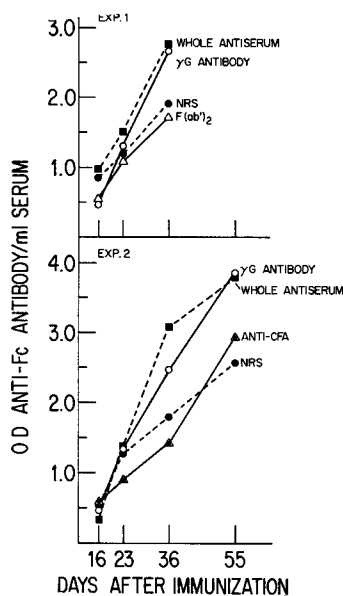


FIG. 4. Anti-Fc antibody produced after active immunization with guinea pig γ_2 -immunoglobulin by rabbits receiving passive anti-F(ab')₂ antibody in different forms. For experimental details see legend to Fig. 3.

enhancing effect of antiserum to only the γ M class of antibody (13), or to both the γ M and γ G classes (14, 15), experiments were performed to investigate the ability of the γ G fraction of rabbit anti-guinea pig F(ab')₂ antiserum to enhance the immune response against the Fc region of guinea pig γ_2 -immunoglobulin. In addition, to determine whether the enhancing effect is dependent upon the Fc portion of the antibody molecule, the ability of the F(ab')₂ fragments of the γ G antibody to enhance anti-Fc antibody formation was also explored. The composite data presented in Figs. 3 and 4 are the results of two experiments in which different doses of rabbit anti-guinea pig F(ab')₂ antibodies, or their pepsin fragments, were used. In both experiments whole antiserum or the γ G-immunoglobulin derived from it (containing equal amounts of anti-F(ab')₂ antibody) suppressed anti-F(ab')₂ antibody synthesis (Fig. 3)

and enhanced the anti-Fc response (Fig. 4). As noted also in the previous experiments, the enhancement effect became evident at a later time after immunization (day 36) than did suppression (day 16 or earlier).

Interestingly, the $F(ab')_2$ fragments of the γG antibody also caused suppression of the anti- $F(ab')_2$ response (in accord with the previous findings in references 16–19), but did not show evidence of having caused enhancement of anti-Fc antibody synthesis at any of the times tested. In this instance, a total of 20 mg of antibody fragments were injected by eight daily injections, to try to compensate for the short half-life of circulating rabbit $F(ab')_2$ fragments (20).

Specificity of the Enhancement Phenomenon

Suppression has been shown to be specific at the antigenic determinant level (1, 14, 15, 21–25). In contrast, enhancement is nonspecific in the sense that antibodies specific for one portion of a molecule can enhance the production of antibodies directed toward another portion; for example, anti- $F(ab')_2$ antiserum can enhance the production of antibodies against Fc determinants. But while nonspecific, this enhancement of the anti-Fc response most likely stems from the initial specific binding of anti- $F(ab')_2$ antibody to the $F(ab')_2$ region of the guinea pig γ_2 -immunoglobulin molecule. However, an alternative explanation might be that these antisera enhance the immune response entirely nonspecifically, that is, independently of their anti- $F(ab')_2$ antibody content. This is unlikely for the following reasons: (a) Enhancement is mediated by the γG -globulin fraction of the antisera, as shown above. (b) We have previously shown that an anti-dinitrophenyl antiserum could not enhance the response of rabbits to the Fc region of guinea pig γ_2 -immunoglobulin (1). In order to clarify further this point, two experiments were performed.

(a) Since the antigens used for immunizing both the donors and recipients of passive antisera were incorporated in CFA, we wanted to determine whether antibodies directed against components of CFA, or nonspecific serum constituents, might be contributing to the enhancement observed in our experiments. For this purpose, a group of rabbits immunized with guinea pig γ_2 -immunoglobulin (experiment 2, Figs. 3 and 4) was also injected with pooled serum derived from rabbits previously immunized with CFA alone. As Figs. 3 and 4 indicate, this antiserum did not cause enhancement of the response against the $F(ab')_2$ or the Fc regions of γ_2 -immunoglobulin.

(b) In order to exclude the possibility that the enhancement effect is due to a nonspecific enhancing factor in the passive antisera rather than to specific antibody, the ability of antiserum specific for serum IgA to enhance the immune response against an unrelated antigen, chicken ovalbumin, was tested. The anti-ovalbumin response in a group of five rabbits given ovalbumin and

antiserum against serum IgA was not significantly different from the response of five control rabbits receiving ovalbumin and normal rabbit serum.

DISCUSSION

The present studies represent an attempt to clarify the mechanisms through which passively administered antibody may simultaneously enhance and suppress the immune response against different antigenic determinants on the same molecule. The significant new observations which have been made can be summarized as follows:

(a) Our previous findings with guinea pig γ_2 -immunoglobulin as antigen in rabbits (1) have been confirmed using an analogous but different system of antigen and antibodies. In the present studies, human secretory IgA was used as an antigen in rabbits which received passively serum from rabbits immunized with human serum IgA. A significant enhancement of antibody synthesis against the secretory component portion of the secretory IgA molecule was achieved, while antibody production against the portion common to both serum and secretory IgA, i.e. α and L chain determinants, was reduced in comparison with that of control animals.

(b) In both systems used (guinea pig γ_2 -immunoglobulin + anti-F(ab')₂ antibody, and human secretory IgA + anti-serum IgA antibody), the timing for achieving maximum suppression or enhancement of antibody production was not the same. The suppressive effect was first noted and most marked early in the immune response, whereas the enhancement phenomenon was evident only much later. This difference, which has not to our knowledge been pointed out before, may be one of the reasons for some conflicting results in the literature, and suggests that suppression and enhancement may be independent phenomena.

(c) Other findings also suggest that the mechanisms of the suppression and enhancement phenomena may be unrelated: (i) The fact that enhancement of the anti-Fc antibody response could be obtained with doses of anti-F(ab')₂ antibody that did not cause suppression of anti-F(ab')₂ antibody synthesis suggests that enhancement of the response to certain determinants on a molecule is not dependent upon the simultaneous discernible suppression of the response to other determinants on the same molecule. (ii) While the F(ab')₂ fragments of rabbit antibody directed against guinea pig F(ab')₂ fragments could still mediate suppression of the immune response to the homologous antigenic determinants (see also 16-19), no enhancement effect toward the Fc region of the guinea pig γ_2 -immunoglobulin molecule was observed. These findings might be questioned on the basis of the higher catabolic rates of the antibody F(ab')₂ fragments when injected into rabbits (20). It should be pointed out, however, that other results with the same antigen-antibody system (see

Results, Paragraph 5) demonstrated that enhancement can be obtained with smaller doses than those needed for suppression. Therefore, the higher catabolic rates of the $F(ab')_2$ fragments, leading to their fast elimination, should affect suppression more readily than enhancement, yet this is the opposite of what has been observed. Thus, it seems reasonable to conclude that enhancement, but not suppression, is Fc dependent. It remains to be determined whether the importance of the Fc region for enhancement stems from its role in the concentration of antigen in strategic regions of the lymphoid organs (26–28) or from some other unknown mechanism.

(d) In view of previous findings on the relative importance of different classes of antibodies for enhancement and suppression (13, 14, 29–33), it is of interest that in our experiments the γ G fraction of antisera was capable of mediating both effects. Since our γ G preparations contained no detectable γ M-immunoglobulin, these results appear to rule out the possibility of an exclusive role for γ M antibody in the enhancement phenomenon (see also 14). It still remains possible, however, that on a molar basis γ M-immunoglobulin may prove to be a more effective mediator of the augmentation of the immune response.

(e) Finally, the possibility that the enhancement effect observed after the passive administration of antiserum is initiated by a serum factor other than antibody seems unlikely for the following reasons: (i) It can be obtained with the DEAE-purified γ G fraction of the antiserum. (ii) It is not mediated by the serum of rabbits immunized with CFA alone. (iii) We have previously shown that an antiserum obtained from rabbits immunized with dinitrophenylated-bovine gamma globulin does not enhance the response of rabbits against the Fc fragment of guinea pig γ_2 -immunoglobulin (1); and (iv) in this study we found that an antiserum specific for human serum IgA, capable of enhancing the immune response to the secretory component of human secretory IgA, did not significantly enhance the response of rabbits to ovalbumin.

In summary, it appears that our findings can best be explained by the hypothesis proposed by Pearlman (14) that antibodies suppress specifically the immune response to determinants to which they can bind, and simultaneously enhance the response to other determinants on the same molecule in a “nonspecific” manner. According to this view, the ability of an antiserum of heterogeneous specificity to cause discernible suppression or augmentation of the response against a multideterminant antigen may actually represent the net balance of these two effects at the determinant level (see also 14, 34).

With the exception of certain findings such as those of Henney and Ishizaka (35) and of Cerottini et al. (24), which are in contradiction with some of the data presented above, many other observations on the enhancing effect of passive antibody (14, 15, 25, 34) agree with our findings. Furthermore, many examples are known of the importance of prior or simultaneous active im-

munization to some (presumably stronger) determinants in the induction of an antibody response against other weakly immunogenic determinants on the same molecule (36–41) or cell (42, 43), and in the induction of transplantation immunity against adjacent weak cell surface antigens (44–47). Some of these effects are referred to as “hapten–carrier relationships” or “cooperation of antigenic determinants.”

From the recent studies of Mitchison et al. (48–50), Rajewsky et al. (51), Katz et al. (52), and Paul et al. (53), there has arisen the hypothesis that this effect is mediated by the interaction of carrier-specific and hapten-specific cells. Their findings suggest that cell-bound receptors rather than circulating anti-carrier antibodies play an important role in augmenting the response to haptenic determinants, since in none of these studies could passively administered anti-carrier antiserum substitute effectively for active immunization to the carrier (40, 51, 52) or adoptive transfer of carrier-primed lymphocytes (48, 53) in enhancing the anti-hapten response.

Nonetheless, what may appear to be two different mechanisms for augmentation of the immune response, i.e. augmentation induced by circulating antibody or by cell cooperation, may yet prove to be a reflection of the same immunologic phenomenon. It may be, for example, that the powerful enhancing effect of carrier-sensitized cells, in comparison with the poor activity of serum antibodies, is due to the specific localization of the sensitized cells in certain areas of the lymphoid organs where secreted antibody might be more effective. Alternatively it is possible that under certain circumstances cell-bound antibody may be released into the circulation and cause the localization of antigen at the surface of other cells. There is a good precedent for this reasoning in the case of anaphylactic antibody, for it is well known that although it may be quite easy to sensitize an animal for anaphylactic shock (and in this case anaphylactic antibody, produced by lymphoid cells, is bound to mast cells), it is much more difficult to demonstrate circulating reagin (IgE). In any case, whatever its mechanism, our results demonstrate that intramolecular augmentation of the immune response can be used effectively toward the practical goal of providing strong antisera against molecular determinants that are but weakly antigenic when immunization is carried out by more conventional schemes.

SUMMARY

The ability of passively administered antibody to suppress the immune response against homologous antigenic determinants while concomitantly enhancing the response against other unrelated determinants of the same antigen molecule has been established in two distinct antigen–antibody systems: (a) guinea pig γ_2 -immunoglobulin + passive anti-F(ab')₂ antibody, where suppression of anti-F(ab')₂ antibody synthesis is accompanied by enhancement

of the anti-Fc response; and (b) human secretory IgA + passive anti-serum IgA antibody, where suppression of antibody production against the α and L chains accompanies augmentation of the response to the secretory component.

The mechanisms of the suppressive and enhancing effects are probably unrelated for the following reasons: (a) Enhancement of the response to certain determinants may be obtained without discernible suppression of the response to the homologous determinants; and (b) the F(ab')₂ fragments of passive antibody can mediate immune suppression but were not observed to enhance the response against the unrelated determinants of the same antigen molecule. Also, the timing for achieving maximum suppression or enhancement of antibody formation is not the same; enhancement was obtained only at a later time.

Both the enhancement and suppressive effects were obtained with the purified γ G fraction of antisera. This finding rules out an exclusive role of γ M antibody in the enhancement phenomenon.

We thank Dr. E. C. Franklin for the ascitic fluid containing myeloma protein, and Doctors C. Griscelli and P. Vassalli for the human colostrum. We are also grateful to Dr. Paulo Mello Freire for the statistical analysis.

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