IMMUNODEVIATION BY PASSIVE ANTIBODY, AN EXPRESSION OF SELECTIVE IMMUNODEPRESSION

II. Action of Guinea Pig IgG1 and IgG2 Anticarrier Antibodies

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The phenomenon of immune deviation (1), also termed split tolerance (2) or contrasensitization (3), seems to be of crucial importance in trying to understand the relations between the two phenomena of immunological facilitation (enhancement) and immunological tolerance (4). Its mechanism is possibly due to a selective immunodepressive action of various immunoglobulin classes of antibodies on active antibody production of various classes. The preceding article has indeed shown the differential effect of passive IgG1 and IgG2 antihapten antibodies on the antibody production of these two classes, the main finding being the delayed enhancing effect of passive IgG1 on their own production.[1]

On the other hand, hapten-carrier relationships have been reported in several immunological systems and, more recently, interpreted within the frame of T cell-B cell cooperation (reviewed in 5). Immunization of the guinea pig with a highly hapten-substituted carrier protein leads to antibody production against the hapten and delayed hypersensitivity to the carrier moiety (6). It has therefore been thought of interest to look for the possible effects of passive IgG1 and IgG2 anticarrier antibodies on the active IgG1 and IgG2 antihapten response. The present experiments carried along with the precedingly reported ones[1] deal mainly with these effects.

As it will be seen, the observed results are best explained by postulating a regulatory function of the Fc portion of the IgG1 anticarrier antibody, combined with the antigen, on the T cell.

Materials and Methods

Animals.—Antibody donors and recipients were adult Hartley guinea pigs, of the same origin and kept together with those used in experiments reported elsewhere.[1]

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[1] Vuagnat, P., T. Neveu, and G. A. Voisin. 1973. Immunodeviation by passive antibody, an expression of selective immunodepression. I. Action of guinea pig IgG1 and IgG2 antihapten antibodies. *Eur. J. Immunol.* In press.

Antigens.—Bovine gamma globulin (BGG)[2] and bovine serum albumin (BSA) were dinitrophenylated by the method of Eisen (7). The conjugated proteins were purified by acid precipitation or by filtration on Sephadex G-25. The same preparation of DNP_{51}-BGG and DNP_6-BSA was used throughout the experiments. Subscripts refer to the average number of dinitrophenyl (DNP) groups per protein molecule, calculated from the absorbance at 360 nm and the dry weight.

Human gamma globulin (HGG) was separated from Cohn fraction II by DEAE-cellulose chromatography, using a 0.01 M Tris-HCl buffer, pH 8.0.

Passive Antibodies.—Guinea pigs were immunized as described by Binaghi (8) and bled on day 30. The sera were pooled and decomplemented by heating 30 min at 56°C, and aliquots were adsorbed on polymerized HGG (9). Anti-HGG antibodies were eluted with 0.1 M glycine-HCl buffer, pH 2.8, with a yield of 59%. Pooled eluates were chromatographed on a DEAE-cellulose column equilibrated with 0.004 M phosphate buffer, pH 7.40. After collection of the IgG2 in the effluent, the IgG1 were eluted with a linear NaCl gradient. The fraction eluted between 0.04 and 0.1 M NaCl was selected.

After concentration to 9 mg/ml, both IgG1 and IgG2 fractions had a titer of 1:10,000 by passive hemagglutination. The IgG2 fraction had a titer of 1:16,000 by passive hemolysis, and of 1:5 by passive cutaneous anaphylaxis (PCA), although no IgG1 could be antigenically detected by the Ouchterlony technique. The IgG1 fraction has a PCA titer of 1:8,000 and a hemolytic titer of 0, even though some IgG2 could be antigenically detected.

The relative avidity of the IgG1 and IgG2 antibodies was found to be similar, using the method described by Cerottini et al. (10). IgG1 and IgG2 antibodies were used at the concentration of 2 μg/ml of 0.15 M borate buffer, pH 8.2, containing a 1:15 dilution of a hyperimmune guinea pig antiovalbumin serum as carrier. [^{125}I]HGG (22 nCi/μg) was obtained according to the method of McConahey and Dixon (11). After overnight incubation at 36°C in a shaking water bath and sampling, a 300-fold excess of cold HGG was added. Aliquots were sampled at given intervals thereafter, starting on the 10th min. IgG1- or IgG2-bound radioactivity was determined by precipitation with a preheated anti-IgG1 or anti-IgG2 monospecific rabbit serum. All determinations were done in duplicate, along with the necessary controls.

Finally, sheep red blood cells were coated with dinitrophenylated bovine gamma globulin (DNP_{51}-BGG) by the chromium chloride method (12). A 1.5‰ concentration of $CrCl_3$ was found to be adequate. With this antigen, anti-HGG IgG2 were three twofold dilutions less effective in the passive hemagglutination test than purified anti-BGG IgG2 used at the same concentration.

Immunization of Experimental Animals.—This was done under the same conditions and at the same time as the immunization of the animals used in previous experiments.[1] Allocation of animals into groups homogeneous for sex and weight, and allocation of treatments, was randomized. Adequate quantities of DNP_{51}-BGG and of antibody preparations were incubated 60 min at 37°C in a shaking water bath; then the whole mixture was emulsified with an equal volume of complete Freund's adjuvant (Difco Laboratories, Detroit, Mich.). Each animal received 0.2 ml of emulsion containing 25 μg of antigen and 500 μg of antibody, divided between the hind footpads. A booster injection of 100 μg of antigen in saline was given on the 12th wk, divided into 6 intradermal sites on the shaved back.

Determination of Active Responses.—Serum antidinitrophenyl (DNP) IgG1 and IgG2 antibody contents were measured by the modified Farr method, using the same reagents and the same conditions as those described elsewhere.[1] Actually, sera from both experiments

[2] *Abbreviations used in this paper:* BGG, bovine gamma globulin; BSA, bovine serum albumin; DH, delayed hypersensitivity; HGG, human gamma globulin; PCA, passive cutaneous anaphylaxis.

were assayed at random and blindly, irrespective of the treatment a given animal had received. All test sera from any one animal were assayed on the same day.

Delayed hypersensitivity to the carrier was assessed by skin test in four other animals of each group (nine animals in the control group receiving antigen alone) on the 5th wk after primary immunization, i.e., at the peak of the antihapten antibody response. 10 and 50 μg of BGG were injected intradermally 24 h, and Evans blue intravenously 100 min, before sacrifice.

RESULTS

Effect of Passive Anticarrier Antibodies on the IgG2 Antihapten Response.—As shown in Fig. 1, passive IgG2 had a unique effect the 1st wk after immunization, the diminution of the antihapten IgG2 antibody level being highly significant

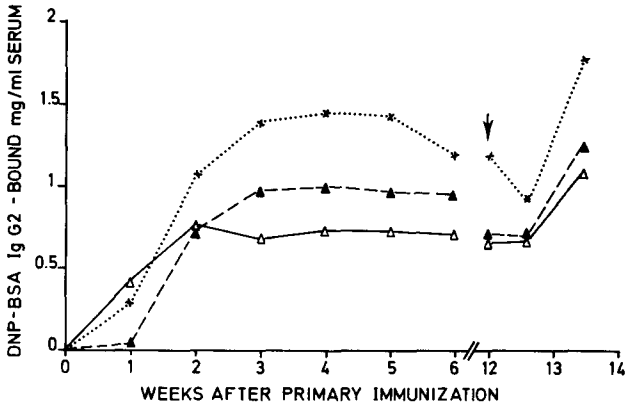

FIG. 1. Groups of six guinea pigs were immunized on day 0 with 25 μg of DNP_{51}-BGG either alone (×·····×) or mixed with 500 μg of purified anti-HGG IgG1 (△——△) or IgG2 (▲----▲) antibodies. A booster injection of 100 μg of antigen in saline was given on the 12th wk (arrow) and serum was obtained 4 and 10 days later. Active production of anti-DNP IgG2 antibodies was assayed by the Farr method, using an anti-IgG2 monospecific rabbit serum. Individual determinations were made in duplicate and the mean computed for each group.

($P < 0.005$, Student's t test). From the 2nd wk onward however, the IgG2 response, although reduced, was the least suppressed of all groups receiving passive antibodies, either antihapten or anticarrier.

On the other hand, passive immunization with anticarrier IgG1 antibodies reduces more effectively the IgG2 antihapten response, and this to a degree strikingly similar to the one observed after passive antihapten IgG1 antibodies and reported elsewhere.[1] The latter point is illustrated in Fig. 2.

Effect of Passive Anticarrier Antibodies on the IgG1 Antihapten Response.—Unexpected was the absence, from the 2nd wk onward, of a suppressive effect of passive anticarrier IgG2 antibodies on this response, as illustrated in Fig. 3.

Passive anticarrier IgG1 antibodies however are as suppressive as were passive antihapten IgG2 antibodies, and this effect is still seen, although not signifi-

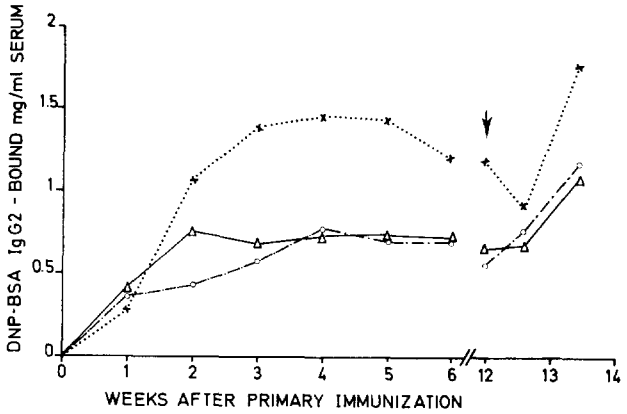

FIG. 2. Groups of six guinea pigs were immunized on day 0 with 25 μg of DNP_{51}-BGG either alone (×·····×) or mixed with 500 μg of purified anti-HGG IgG1 (△——△) or anti-DNP IgG1 (○—·—·○) antibodies. A booster injection of 100 μg of antigen in saline was given on the 12th wk (arrow). This graph is a combination of results presented in Fig. 1 and elsewhere.[1]

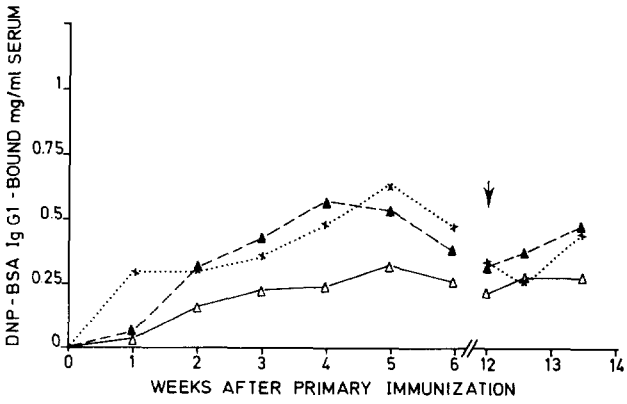

FIG. 3. Groups of six guinea pigs were immunized on day 0 with 25 μg of DNP_{51}-BGG either alone (×·····×) or mixed with 500 μg of purified anti-HGG IgG1 (△——△) or IgG2 (▲---▲) antibodies. A booster injection of 100 μg of antigen in saline was given on the 12th wk (arrow), and serum was obtained 4 and 10 days later. Active production of anti-DNP IgG1 antibodies was assayed by the Farr method, using an anti-IgG1 monospecific rabbit serum. Individual determinations were made in duplicate and the mean computed for each group.

cant, 10 days after reimmunization. This is the moment (Fig. 4) when the dissociation between the effects of passive IgG1 anticarrier and antihapten antibodies on the IgG1 antihapten response is most obvious ($P < 0.0005$). This dissociation is one of the bases for the interpretation given in the discussion.

The data, with regard to the effect of passive anticarrier antibodies, are summarized in Table I.

Effect of Passive IgG1 and IgG2 Antibodies on Delayed Hypersensitivity.— With the doses of antigen used to skin test the animals on the 5th wk after primary immunization, no clear-cut differences were seen between the experimental groups, whether they had received passive IgG1 or IgG2 anticarrier or antihapten antibodies, although all appeared to be somewhat inhibitory.

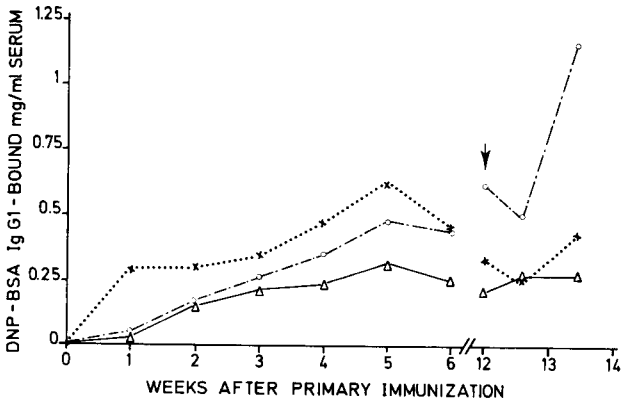

FIG. 4. Groups of six guinea pigs were immunized on day 0 with 25 μg of DNP_{51}-BGG either alone (×···×) or mixed with 500 μg of purified anti-HGG IgG1 (△——△) or anti-DNP IgG1 (○—·—·○) antibodies. A booster injection of 100 μg of antigen in saline was given on the 12th wk (arrow). This graph is a combination of results presented in Fig. 3 above and elsewhere.[1]

TABLE I

*Action of Passive Anticarrier Antibodies on the Humoral Antihapten Antibody Response After Primary and Secondary Immunization**

Passive anti-HGG antibodies used	Active anti-DNP response‡			
	IgG2		IgG1	
	Primary	Secondary	Primary	Secondary
IgG2	56§	71	94	109
IgG1	60‖	62§	50‖	64

* Primary immunization consisted of 25 μg of antigen emulsified in complete Freund's adjuvant with 500 μg of antibody. Antigen alone in saline was used to boost the animals on the 12th wk (secondary immunization).

‡ The primary response is expressed as its surface area until the 6th wk, relative to controls taken as 100. Results were compared by Wilcoxon's test. The secondary response is that of day 10 after boosting, relative to controls taken as 100. Results were compared by Student's *t* test.

§ $P \leqslant 0.05$.

‖ $P \leqslant 0.005$.

DISCUSSION

The main finding of the present study is the sustained suppressive effect of passive IgG1 anticarrier antibodies on the active IgG1 antihapten response, as opposed to the delayed enhancing effect of passive IgG1 antihapten antibodies.

Being available in purified form and of comparable relative avidity, IgG1 and IgG2 anti-HGG antibodies were used as anticarrier antibodies, since they are directed against a restricted number of determinants of the carrier moiety of the DNP-BGG molecule. Theoretically, the ideal system would be to use two different haptens, each one being carrier for the other; but this in the guinea pig does not allow delayed hypersensitivity (DH) to be studied. Furthermore, haptens do not seem to function as carriers in this species (13). They may do so in the mouse (14), and this would not be surprising if haptens induce DH in the mouse as they do in the rat (15; T. Neveu, unpublished observations).

Indeed, the use of complete Freund's adjuvant for sensitization in the studies on the carrier effect induces DH to the carrier (13, 16, 17). Carrier and hapten determinants are unrelated, separate determinants (14, 17); this conclusion is strongly favored by experiments where tolerance to the carrier reduces the response to the hapten (13, 18, 19). Carrier and hapten determinants must be on the same molecule (14, 17, 20), although recent reports challenge this conclusion (21–24).

Nonetheless all reports suggest that two separate populations of cells cooperate, anticarrier thymus-derived T cells and antihapten non-thymus-derived B cells. How these two populations cooperate is not yet settled. In order to be activated, the B cell apparently needs more than one signal: two or more molecules of antigen cross-linked by anticarrier antibody (25) or by anticarrier T cells, these being then linked to the B cell through the antigen (26). In some instances, however, the second signal does not originate in the antigen under study; T cells might act on B cells either directly as in the allogeneic effect (22, 23) or through the liberation of a mediator induced by their interaction with any antigen to which they are sensitized (21, 24). These interpretations must be accepted with caution, inasmuch as they are inferred from complex systems in which cross-reactivity between antigens might furthermore exist. This, for example, seems to be the case of sheep RBC and horse RBC (27), the system used by Hartmann (21). Finally, the recent report of Gershon and Kondo, which suggests that T cells might produce a "shut off" substance (28), makes any current interpretation even more difficult.

On the other hand, a cooperating role of anticarrier antibody appears to be invalidated by several experiments (16, 17, 29). When given passively, anticarrier antibodies either have no effect (6, 29) or yield a considerable depression of the antihapten response (16, 30).

This has been our experience in the present study. Yet the IgG1 antihapten response was normal after passive IgG2 anticarrier antibodies and depressed

after passive IgG1 anticarrier antibodies. This apparent discrepancy could not be accounted for by a difference in the dissociation rate of these two antibody classes from immune complexes.

The interpretation we favor at this time is the following: Antibody would influence antigen localization through its class and then, at least for IgG1 antibodies, influence the immune system according to its specificity, either antihapten or anticarrier.

Many observations substantiate these assumptions. Guinea pig IgG2 antibodies are cytophilic for macrophages and the strength of the binding is considerably increased when the antibody has reacted with the antigen (31). Similarly, mouse B cells have a receptor exhibiting a marked predilection for IgG1 antibody, the bond being stabilized when the antibody has reacted with the antigen (32, 33). Furthermore, only IgG1 antibodies have been shown to be highly thymus dependent in the mouse (34, 35). There are many similarities between mouse and guinea pig IgG1 antibodies, such as the triggering of passive cutaneous anaphylaxis (36, 37); these similarities justify the assumption that guinea pig IgG1 antibodies are thymus dependent too.

Immunization with IgG2-antigen complexes will lead to antigen removal by the macrophages. Our observation that anticarrier IgG2 antibodies were less suppressive than antihapten IgG2 antibodies may be explained by the fact that only part of the few available determinants of our highly hapten-substituted carrier protein could combine with the cross-reacting anticarrier antibodies we used.

Immunization with IgG1-antigen complexes, on the other hand, will promote the persistence of the antigen in the immune centers. Antihapten IgG1 antibody will mask the haptenic determinants, but at the same time will allow sensitization to the carrier, as suggested by Tada and Okumura (38). This, together with the thymic dependency of IgG1 antibodies, would account for the brisk increase of antihapten IgG1 antibodies when the antigen is reintroduced. As for anticarrier IgG1 antibodies, they would shut off carrier-specific T cells, this resulting in a depressed antihapten antibody response, even after reimmunization, as can be noticed for the IgG1 response.

This interpretation, akin to the "tripartite inactivation model" proposed by Sinclair et al. (39), gives a regulatory function to IgG1 antibodies, as suggested by Crowle and Hu (40), and further reconciles the two main alternatives, peripheral vs. central, which have been put forward to explain the role of antibody in the regulation of the immune response (41). It will doubtless require modification as new evidence comes to light, but it would seem for the present to be a convenient summary of significant information. Such a scheme at least emphasizes the delicate balance and the dynamic intricacies between available committed cells, antigenic determinants, and antibodies of the different classes at any time during the immune response.

SUMMARY

The effect of passively administered IgG1 and IgG2 anticarrier antibodies on the IgG1 and IgG2 antihapten response has been studied.

Guinea pigs were immunized with dinitrophenylated bovine gamma globulin mixed with purified IgG1 or IgG2 antihuman gamma globulin antibodies, i.e., antibodies directed against a limited range of the carrier determinants. Humoral IgG1 and IgG2 anti-DNP antibody contents were assayed at weekly intervals and 4 to 10 days after a booster injection of antigen in saline given on the 12th wk.

The main finding was the sustained suppressive effect of passive IgG1 anticarrier antibodies on the active IgG1 antihapten response.

This result is compared with the enhancing effect of passive IgG1 antihapten antibodies and is discussed in the light of T cell-B cell and hapten-carrier relationships, leading to the proposal of a regulatory function of the Fc portion of the IgG1 anticarrier antibody, combined with the antigen, on the T cell.

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