SELECTIVE SUPPRESSION OF DELAYED HYPERSENSITIVITY BY THE INDUCTION OF IMMUNOLOGIC TOLERANCE*

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(Received for publication 24 November 1965)

The relationship between delayed hypersensitivity and antibody formation is not yet clear, although the subject has been studied extensively. While delayed hypersensitivity and antibody formation could be phases of the same process, some data suggest that these two phenomena may be fundamentally different and mediated by separate mechanisms. For example, it has been shown that the acquisition of delayed hypersensitivity can be suppressed by the antimetabolite 6-mercaptopurine (6-MP) without a concomitant suppression of antibody formation (1–3). In agammaglobulinemia humoral antibody synthesis is markedly impaired, while delayed hypersensitivity may be expressed normally (4). The reverse is true in Hodgkin's disease: the formation of circulating antibodies is normal, but delayed hypersensitivity reactions are profoundly depressed (5). There is thus some evidence suggesting that delayed hypersensitivity and antibody formation are not necessarily directly linked together.

The present experiments were undertaken in order to investigate whether delayed hypersensitivity and antibody formation are equally affected by the induction of immunologic tolerance. By this approach, it was found that guinea pigs could be rendered tolerant to hapten protein conjugates with respect to delayed hypersensitivity only, without concomitant impairment of antibody formation to the same antigen.

Materials and Methods

Animals.—Outbred newborn guinea pigs and adult animals weighing 400 to 600 g were used for most of these experiments. Albino guinea pigs weighing 250 to 350 g were used for passive cutaneous anaphylaxis.

Antigens.—2,4-Dinitrophenylsulfonic acid (DNPS) (Eastman Kodak Co., Rochester, New York) twice recrystallized was used for coupling to 3 different carrier proteins: bovine gamma globulin (Armour Pharmaceutical Co., Kankakee, Illinois), crystalline bovine serum albumin (Armour), and rabbit gamma globulin (Pentex, Inc., Kankakee, Illinois). *O*-Chlorobenzoyl chloride (Eastman Kodak) was coupled to bovine gamma globulin.[1]

* This work was supported by grants No. A-3777 and A-4819 of the United States Public Health Services.

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[1] The following abbreviations are used throughout the paper: DNP, dinitrophenyl; DNP-

Conjugates.—DNPS was conjugated according to the method of Eisen (6), as modified by Ovary and Benacerraf (7). After extensive dialysis against distilled water buffered at pH 8 with potassium carbonate, the protein concentration of conjugate was determined by the Folin method, and the average number of hapten groups per molecule of protein conjugate was calculated from its optical density, related to the extinction coefficient of dinitrophenyl at 360 μ. Two series of experiments were conducted, one with preparation I, containing 37 groups of hapten per mole of carrier (37 groups/mole), and one with preparation II, containing 32 groups/mole. The same antigen was used to immunize and challenge the animals. DNPS was similarly conjugated to RGG (28 groups/mole) and to BSA (36 groups/mole). *O*-Chlorobenzoyl chloride was conjugated to BGG according to the method of Benacerraf (8).

Induction of Tolerance.—Newborn guinea pigs were divided in three groups: *Group I*, 20 newborn guinea pigs were injected twice i.p. with 4 mg of DNP-BGG, given on the day of birth and 1 wk later. *Group II*, 15 newborn guinea pigs were injected only once i.p. with 4 mg of DNP-BGG on the day of birth. *Group III*, 15 control littermates were not injected with the antigen during the 1st wk of life.

At 4 wk of age all animals, control and experimental, were immunized with 200 μg of DNP-BGG in complete Freund's adjuvant in all foot-pads. At the same time 200 μg of OCBC-BGG in complete adjuvant was injected subcutaneously.

Eleven adult guinea pigs were pretreated with 2 i.p. injections of DNP-BGG given 7 days apart at either the same dose (group IV) as the newborn animals, or at a dose adjusted to body weight so that they received the same amount per kilogram of body weight as the newborn guinea pigs (group V). All adult animals were similarly immunized with 200 μg of DNP-BGG in complete adjuvant in all foot-pads and with 200 μg of OCBC-BGG in complete adjuvant subcutaneously.

Skin Tests.—The animals injected neonatally, as well as control littermates, were skin tested on the 14th day after the last injection of antigen. The animals were carefully shaved on their flank and 50 μg of the antigens (DNP-BGG, DNP-RGG, BGG, and OCBC-BGG) were injected intradermally in a volume of 0.1 ml of normal saline solution. The animals were observed at 2, 4, 6, and 24 hr and the nature of the reactions, immediate or delayed, was differentiated and graded. Immediate reaction at 3 hr was graded as follows: +, slight edema without hemorrhage; ++, moderate to severe edema with or without slight hemorrhage; and +++, severe edema with hemorrhage. Delayed reaction at 24 hr: +, induration 8 to 10 mm in diameter; ++, induration 11 to 12 mm in diameter; and +++, induration 13 to 15 mm in diameter.

Arthus reactions appeared within 1 hr after challenge and were at their maximum intensities at 2 to 3 hr. Delayed reactions appeared after 6 hr and reached their maximum intensities at 18 to 24 hr. These reactions were generally more severe than the immediate ones and consisted of erythema and induration, occasionally with central necrosis. The adult animals were skin tested on the 7th day after the immunizing dose; the doses of antigen used to skin test were 10 and 50 μg.

Antibody Determination.—Animals of group I were bled by intracardiac puncture at 4 wk of age prior to immunization and all animals were bled by intracardiac puncture performed 24 hr after each skin test; the following tests were used for the detection of antibodies.

Passive hemagglutination: A modification of the micromethod of Heller et al. (9) was used.

BGG, dinitrophenyl bovine gamma globulin; DNP-RGG, dinitrophenyl rabbit gamma globulin; DNP-BSA, dinitrophenyl bovine serum albumin; OCBC, *O*-chlorobenzoyl chloride; OCBC-BGG, *O*-chlorobenzoyl chloride bovine gamma globulin; BGG, bovine gamma globulin; BSA, bovine serum albunin; RGG, rabbit gamma globulin; HGG, human gamma globulin; and PCA, passive cutaneous anaphylaxis.

If a preparation of DNP-BGG other than the one employed for the induction of tolerance was used, the number of groups per mole was in the same range as the original antigen, and in this instance a reference serum was also tested to insure that comparable titers were obtained with the 2 different preparations of DNP-BGG. In some experiments absorption studies were carried out as follows: antibodies were assayed against DNP-BGG, DNP-RGG, and BGG in (*a*) native serum, (*b*) serum absorbed with the whole original antigen, DNP-BGG, (*c*) serum absorbed in antigen excess with DNP-RGG and BGG, and (*d*) supernatant after precipitation of the antihapten antibody with DNP-RGG and then absorbing the resulting supernatant with the carrier protein BGG. The absorptions were done overnight at 4°C with 0.2 ml of DNP-BGG (1.25 mg of protein/ml), for (*b*) or 0.2 ml of serum and 0.2 ml of DNP-RGG (1.25 mg of protein/ml) and 0.2 ml BGG (1.25 mg of protein/ml) for (*c*). For (*d*), 0.5 ml of serum and 0.1 ml containing the amount of DNP-RGG necessary to precipitate the total amount of anti-DNP antibody was incubated 1 hr at 37°C and then overnight at 4°C. The precipitate was spun down and removed, and the resulting supernatant was then absorbed with an equal amount of BGG (1.25 mg of protein/ml) as described above. These absorbed sera were tested in double diffusing agar plates against a rabbit serum anti-BGG and a guniea pig serum anti-DNP-BSA to insure that they were carried out in antigen excess.

Passive hemolysis: The procedure described by Kabat and Mayer (10) as modified by Bloch (11) has been used for this purpose. In addition to the control test referred to (11), heat-inactivated guinea pig serum was used in order to compare the titer of the passive hemagglutination with the titer of passive hemolysis. This test was done with the native serum and the supernatant after precipitation with DNP-RGG and absorption with BGG using tanned cells coated with DNP-BGG as the antigen.

Passive cutaneous anaphylaxis: The method described by Ovary was used (12). In the qualitative PCA a serum dilution of 1/100 and 250 μg of the antigen (DNP-BGG) were used. In the quantitative PCA for absorption studies serial serum dilutions were done. Absorbed and nonabsorbed serum were used in the same animal: (*a*) native serum; (*b*) serum absorbed with DNP-BGG (antigen used for immunization); and (*c*) serum was precipitated with DNP-RGG in slight antigen excess as described in the paragraph of passive hemagglutination. After removal of precipitate the supernatant was absorbed with BGG. Three animals were used for each antigen (DNP-BGG, DNP-RGG, and BGG).

Agar gel diffusion studies: Antibody activity against DNP-BGG, DNP-RGG, and BGG was also studied by agar gel double diffusion using the Petri dish method of Ouchterlony (13)

RESULTS

Delayed Hypersensitivity.—It was found that the majority of guinea pigs did not exhibit delayed hypersensitivity to an antigen if they had been injected twice in the neonatal period with this antigen: 10 of 17 animals of group I (injected twice neonatally with DNP-BGG) did not produce a positive delayed reaction to this antigen after subsequent immunization in complete adjuvant. On the other hand, all animals of this group had a positive skin reaction to OCBC-BGG, the antigen given at the age of 4 wk (Table I). All animals of group III (control littermates not injected neonatally) but immunized in complete adjuvant with DNP-BGG and OCBC-BGG, had a positive skin test to DNP-BGG and all but one to OCBC-BGG (Table II). Injection of the antigen only once at birth did not result in the suppression of the 24 hr skin reaction at 6 wk of age in a large proportion of animals: 8 out of 12 animals of group II produced a positive skin reaction to DNP-BGG while again all animals developed

a positive skin test to the second antigen OCBC-BGG. The result of the skin tests with BGG, i.e. the carrier protein, was similar to that of the skin tests with DNP-BGG.

In contrast, administration of DNP-BGG to adult guinea pigs, either in the same amount as used in group I or with a body weight adjusted dose, failed to affect the expression of delayed hypersensitivity to DNP-BGG after subsequent immunization in complete Freund's adjuvant. A summary of the results of all these experiments is given in Table III.

TABLE I

24-Hr Skin Tests in Animals of Group I at 6 Wk of Age

Animal No.	DNP-BGG	BGG	OCBC-BGG
6-03	−*	+	++
6-06	−	−	+
6-10	−	−	++
4-52	−	−	+++
1-27	−	−	++
1-37	−	−	++
1-46	−	−	+++
1-50	−	−	+++
3-75	−	++	+++
4-69	−	+	+++
6-05	+	+	+++
4-67	++	−	+++
1-56	++	+	+++
1-57	++	++	+++
4-72	+++	++	+++
4-53	+	+	++
6-92	+++	+	+++

*, negative; +, 6 x 10 mm induration; ++, 10 x 12 mm induration; +++, 12 x 15 mm induration.

In order to determine the duration of the suppression of delayed hypersensitivity to DNP-BGG, repeated skin tests were done in 10 animals in which delayed hypersensitivity was not observed at 6 wk of age. Six animals were skin tested at 2.5 months and 4 at 3.5 months. The results are listed in Table IV.

Circulating Antibodies.—The sera of all animals had circulating antibodies to DNP-BGG, whether or not delayed hypersensitivity to this antigen was detectable. Antibodies were revealed in each animal by passive hemagglutination and PCA. In addition, most of the sera of animals of all groups gave a precipitin line in the Ouchterlony plates when tested with DNP-BGG (Table V). There was no relation between the titer of hemagglutination antibody and the presence or absence of delayed hypersensitivity or the type of treatment during the neonatal period (Table VI).

TABLE II

24-Hr Skin Tests in Animals of Group III at 6 Wk of Age

Animal No.	DNP-BGG	BGG	OCBC-BGG
6-04	++*	++	++
6-07	++	++	++
6-25	+++	++	++
6-14	+++	++	−
6-08	+++	++	++
6-02	+++	++	++
1-51	+++	++	+++
1-59	+++	−	+++
4-65	+++	+	+++
1-52	+++	+	+++
1-63	+++	−	++
1-54	+++	−	+++

* See Table I.

TABLE III

24-Hr Skin Tests in the Various Groups

Neonatally injected animals	DNP-BGG	OCBC-BGG
Group I	7/17	17/17
Group II	8/12	12/12
Group III (control littermates)	12/12	12/12
Adult animals		
Group IV	6/6	*
Group V	5/5	5/5

* Not done.

TABLE IV

24-Hr Skin Tests in Animals with a Negative Test at 6 Wk of Age

Time of skin tests	DNP-BGG	OCBC-BGG
months		
2.5	1/6	6/6
3.5	2/4	4/4

Most of the animals of the various groups when challenged with DNP-BGG expressed a mild Arthus reaction (from 1+ to 2+) to DNP-BGG (Table VII) which disappeared after 4 hr and did not interfere with the reading of delayed hypersensitivity. No difference in the intensity of the skin reaction was ob-

TABLE V

Result of Precipitation Test in Agar Plates with Sera against DNP-BGG

Group I	Group II	Group III	Group IV + V
14/17	8/12	12/12	11/11

TABLE VI

Result of Passive Hemagglutination Test with Sera of Animals of Groups I and III, 2 Wk after Immunization with Freund's Adjuvant

Group I				Group III	
Animals with negative 24-hr skin test		Animals with positive 24-hr skin test		Animals with positive 24-hr skin test*	
No.	Reciprocal titer	No.	Reciprocal titer	No.	Reciprocal titer
6-03	1,024	6-05	256	6-04	128
6-06	512	4-67	2,048	6-07	512
6-10	1,024	1-56	2,048	6-25	4,096
4-52	1,024	1-57	2,048	6-14	8,192
1-27	512	4-72	1,024	6-08	1,024
1-37	2,048	4-53	8,192	6-02	128
1-46	1,024	6-92	16,384	1-51	4,096
1-50	1,024			1-59	4,096
3-75	1,024			4-65	2,048
4-69	16,384			1-52	2,048
				1-63	2,048
				1-54	1,024
Mean Titer.....	1,260		2,256		1,367

* No animal of group III had a negative 24 hr skin test.

TABLE VII

Result of Arthus Type Skin Reactions Read at 3 Hr

Group I	Group II	Group III	Group IV + V
15/17	9/12	11/12	11/11

served in the various groups. Similar skin reactions were obtained with DNP-RGG, whereas BGG alone did not produce an Arthus phenomenon.

Three newborn animals of group I bled at 4 wk of age, prior to immunization with DNP-BGG in complete adjuvant, formed antibody to DNP-BGG (reciprocal titer 128, 512, 512) as detected by passive hemagglutination. This

indicates that the antigen given in the neonatal period did not interfere with antibody formation.

All animals had γ_1-antibodies since they were detectable by PCA (14). It was also investigated whether these animals formed complement binding, presumably γ_2-antibodies as detected by passive hemolysis. Two out of 10 sera studied in group I, and 2 out of 6 sera studied in group III, hemolysed the antigen-coated red cells in the presence of complement.

Specificity of the Serologic Reactions.—The specificity of the serologic reactions in terms of carrier protein, hapten, and combined hapten-carrier protein specificity, were investigated by using different antigens in the passive hemagglutination test and by absorption studies. The results of passive hemagglutination tests are listed in Table VIII.

TABLE VIII

Result of Passive Hemagglutination Test for Detection of Serologic Specificity of Circulating Antibodies

Group I			Group II			Group III			Group IV + V		
DNP-RGG*	BGG*	DNP-BGG‡	DNP-RGG	BGG	DNP-BGG	DNP-RGG	BGG	DNP-BGG	DNP-RGG	BGG	DNP-BGG
17/17	14/17	4/12	12/12	12/12	3/5	12/12	8/12	1/6	11/11	11/11	9/11

* Nonabsorbed sera were used against DNP-RGG and BGG.

‡ Sera were absorbed with DNP-RGG and BGG as outlined in Materials and Methods and used against the original antigen DNP-BGG.

Hapten-specific antibodies were detected in all animals by the agglutination of red cells which were coated with the hapten bound to a carrier protein (RGG) unrelated to the one used for immunization. (RGG was shown in preliminary studies not to react with anti-BGG sera.)

Carrier protein-specific antibodies were detected in most of the animals by the use of BGG-coated cells.

Combined hapten-carrier protein specificity was investigated by studying the serologic reactivity of sera which have been absorbed with DNP-RGG (hapten bound to unrelated carrier protein) and with BGG (carrier protein) in antigen excess (see Fig. 1) or in two stages. (See Materials and Methods.) While these absorbed sera no longer agglutinated DNP-RGG- and BGG-coated cells, 17 out of 40 of these sera still agglutinated red cells coated with the original hapten-carrier protein complex (DNP-BGG). The titer was usually 2 steps less than the titer of the absorbed sera. This reactivity could only be removed by absorption with the original antigen DNP-BGG. A typical example is given in Table IX.

In 2 sera which exhibited a combined hapten-carrier protein specificity at 2.5

months of age, this type of specificity could not be detected at 6 wk of age. Although the antibody titer to DNP-BGG was similar at the two different bleedings.

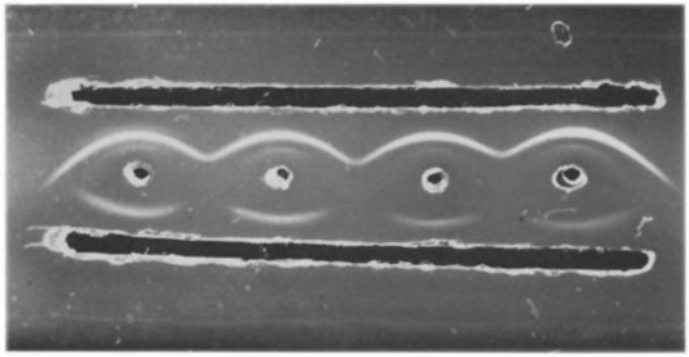

FIG. 1. Demonstration of antigen in the absorbed sera by agar diffusion technique. The 4 center wells contain 4 different sera absorbed with DNP-RGG and BGG. The upper well contains rabbit anti-BGG serum, the lower well guinea pig anti-DNP-BSA serum.

TABLE IX

Passive Hemagglutination Absorption Study with the Serum of Animal 4-59 of Group II with a Negative 24 Hr Skin Test

Treatment of serum	Reversed titer against red cells coated with:			
	DNP-BGG	DNP-RGG	DNP-BSA	BGG
Unabsorbed serum	1024	32	32	128
Serum absorbed with DNP-BGG	—	—	—	—
Serum absorbed with DNP-RGG + BGG	256	—	—	—
Supernatant after precipitation with DNP-RGG and absorption with BGG	256	—	—	—

Out of 10 sera studied in animals with a negative skin test to DNP-BGG (groups I and II), 3 animals' sera had antibody with a combined hapten-carrier protein specificity.

Six sera with a combined hapten-carrier protein specificity as revealed in the passive hemagglutination absorption study were further assayed by PCA and passive hemolysis. (See Table X.)

Native serum, serum absorbed with the complete original antigen, and sera absorbed with hapten conjugated to RGG, and carrier protein as obtained in methods, were assayed against DNP-BGG, DNP-RGG, and BGG. An example showing this combined hapten-carrier protein specificity as detected by PCA is

given in Table XI. By PCA and passive hemolysis all 6 sera had this combined hapten-carrier protein specificity left after absorption of hapten and carrier protein specific antibodies (Table X). It might be coincidental that no serum exhibited this combined specificity in both methods detecting different classes of antibody.

TABLE X

PCA and Passive Hemolysis Assays with 6 Sera Exhibiting a Combined DNP-BGG Specificity in the Passive Hemagglutination Test

The sera have been exhaustively absorbed with DNP-RGG and BGG.

	Group I	Group II	Group IV + V
Animal No.	4-69 6-92	1-60 1-70	1-24 6-83
PCA assay	+ −	+ −	+ +
Passive hemolysis	− +	− +	− −

TABLE XI

Passive Cutaneous Anaphylaxis Absorption Study with the Serum of Animal 1-24 of Group IV with a Positive 24 hr Skin Test

Treatment of serum	Reversed titer against:		
	DNP-BGG	DNP-RGG	BGG
Unabsorbed serum	1600	800	200
Serum absorbed with DNP-BGG	—	—	—
Supernatant after precipitation with DNP-RGG and absorption with BGG	400	—	—

DISCUSSION

The experiments reported here have shown that administration of DNP-BGG to newborn guinea-pigs resulted in the suppression of delayed hypersensitivity to this antigen in more than half of the animals. This suppression was specific and long lasting. In contrast, all animals formed circulating antibodies, whether or not delayed hypersensitivity was present. Delayed hypersensitivity was suppressed in animals treated with the antigen in the neonatal period only and not in adult guinea pigs pretreated with the same amount of antigen per kg of body weight (117 mg of DNP-BGG/kg). Whether suppression of delayed hypersensitivity, as measured by a negative 24 hr skin test, was complete or incomplete cannot be determined. However, it is relevant to note that antibody titers, as detected by passive hemagglutination, were similar in animals in

which delayed hypersensitivity has been suppressed and in animals exhibiting delayed hypersensitivity. These results indicate that a state of tolerance has been induced in terms of delayed hypersensitivity only without affecting antibody formation to the same antigen.

Suppression of delayed hypersensitivity only without suppression of antibody formation to the same antigen may be due to differences in antigenic determinants involved in these two immune phenomena, or alternatively, may indicate that immunologic tolerance in terms of delayed hypersensitivity was induced to one antigenic determinant, without concomitant suppression of antibody formation to the same antigenic determinant. In the first case, one would deal with the induction of tolerance to only part of the antigenic determinants, (those responsible for the 24 hr skin test), and not to others, (those responsible for the serologic tests). Such an immunologic phenomenon would be analogous to what has been referred to as "split tolerance" in transplantation immunity (15). In the second case, delayed hypersensitivity and antibody formation directed to the same antigenic determinant would have been dissociated by the induction of immunologic tolerance.

In order to attack this problem the specificity of delayed hypersensitivity versus the specificity of antibody formation has been further investigated. In all control animals the 24 hr skin test was positive with the original antigen DNP-BGG and in most of the animals with the carrier protein, BGG, alone. It is interesting to note that in most experimental animals in which delayed hypersensitivity to DNP-BGG was suppressed, this skin reaction was also suppressed to the carrier protein alone. The suppression of delayed hypersensitivity to DNP-BGG and BGG was specific, since all control and experimental animals were able to exhibit delayed hypersensitivity to an unrelated antigen OCBC-BGG given together with DNP-BGG in complete adjuvant. The results of the serologic studies in the sera of animals of all groups have shown antibodies with three different specificities: the hapten, the carrier protein, and the combined hapten-carrier protein specificity. Antibody with a combined hapten-carrier protein specificity, i.e. with a specificity similar to that of delayed hypersensitivity to a hapten-carrier protein complex (16), has previously been found in guinea pigs at the onset of the primary immune response only (17). In the present study such antibodies were found also later in the course of immunization. For the question discussed above, it is particularly relevant to note, that such a combined hapten-carrier protein specificity to DNP-BGG was found in animals which did not exhibit delayed hypersensitivity to DNP-BGG. Equally important for this question is the fact that antibody activity against the carrier protein, BGG, alone was present in 8 of 10 animals with a negative 24 hr skin reaction to BGG. Thus, suppression of delayed hypersensitivity to the original antigen (DNP-BGG) as well as to the carrier protein (BGG) was found in animals exhibiting antibody activity with both types of specificities, the combined hapten-

carrier and the carrier specificity. From these results, it appears unlikely that suppression of delayed hypersensitivity was due to induction of tolerance to only part of the antigenic determinants; they rather suggest that delayed hypersensitivity and antibody formation to similar antigenic determinants have been dissociated by the induction of immunologic tolerance.

Reports by others have previously shown that both delayed hypersensitivity and antibody formation were suppressed by administration of antigens to newborn animals (18–20). Turk and Humphrey (18) found that guinea pigs injected prenatally and neonatally with BSA and HGG failed to elaborate circulating antibody and delayed hypersensitivity responses when challenged with these antigens later in life. However, in one group of pretreated animals, immunization with HGG in Freund's adjuvant resulted in antibody production in some of the animals while all animals had a negative 24 hr skin test to HGG. This group of animals may reflect a dissociation of the immune responses similar to the one observed in the present experiment, although the specificities of delayed hypersensitivity and antibody formation were not further investigated.

Administration of antigens to adult guinea pigs prior to immunization with Freund's adjuvant resulted in most of the experiments in the suppression of both delayed hypersensitivity and antibody formation (21–24). However, in some of these studies delayed hypersensitivity has been preferentially affected (22, 23). Thus Chase and Battisto found that prolonged feeding of adult guinea pigs with picryl chloride (PCl) resulted in tolerance to topical application of the hapten; also no antibodies to the hapten were formed in these animals. However, when guinea pigs were immunized with picryl chloride conjugated in vitro to a heterologous protein carrier in Freund's adjuvant, only antibodies to the PCl group were formed, but no contact sensitivity to cutaneous application of PCl could be detected. It should be noted that the specificity of contact sensitivity is known to be directed to a combined hapten autologous carrier skin protein while the specificity of the antibody response is directed to the hapten or the hapten-carrier protein complex used for immunization (25). Thus two different antigenic determinants were involved in contact sensitivity and antibody formation which makes the interpretation of selective suppression of one of the two immune responses difficult.

Experiments reported by Boyden (26) and more recently by Dvorak et al. (24) and Asherson et al. (27) appear to be closely related to the present study. Boyden has found that administration of tuberculoprotein to adult guinea pigs, prior to immunization with BGG, resulted in the diminution of delayed but not immediate skin sensitivity. Dvorak et al. reported that treatment with antigens, administered either prior to or simultaneously with immunization in complete adjuvant, resulted in the suppression of delayed hypersensitivity and complement-fixing antibody. However, γ_1-antibodies were less affected.

Asherson selectively suppressed delayed hypersensitivity without abolishing antibody formation by administration of various antigens before or shortly after immunization with the same antigen in complete adjuvant. In some of the experimental animals the γ_2-antibodies were preferentially suppressed. In the present series of experiments, no qualitative difference in antibody formation was found in control and experimental animals. However, only very few guinea pigs in either group exhibited complement-fixing (γ_2, γ_M) antibody activity.

It is not possible from the present data to determine the exact mechanism underlying the preferential suppression of delayed hypersensitivity. However, the finding that this suppression could be achieved in animals injected in the neonatal period, but not in adult animals, suggests a mechanism similar to that operating in the induction of immunologic tolerance. The mere observation that delayed hypersensitivity can be dissociated from antibody formation by selective induction of immunologic unresponsiveness provides further evidence that these two immunologic processes may be basically different.

SUMMARY

Administration of DNP-BGG to newborn guinea pigs resulted, in more than half of the animals, in the specific suppression of delayed hypersensitivity to DNP-BGG and BGG, as shown after immunization with DNP-BGG in complete Freund's adjuvant.

In contrast, all animals formed antibodies to DNP-BGG, whether or not delayed hypersensitivity to this antigen was present. No difference in antibody titers was found between pretreated and control animals. All animals had antibodies reacting specifically to the hapten DNP, and most of them to the carrier protein BGG, whether or not delayed hypersensitivity to the carrier protein was present. Furthermore, some animals with and without positive 24 hr skin test to DNP-BGG had antibodies with a combined hapten-carrier protein specificity to this antigen, i.e., a specificity which is similar to that of delayed hypersensitivity. Thus, delayed hypersensitivity and antibody formation to similar antigenic determinant were differently affected by injection of antigen in the neonatal period.

The finding that delayed hypersensitivity and antibody formation could be dissociated by the induction of immunologic tolerance supports the assumption that delayed hypersensitivity and antibody formation are different immune processes which are not necessarily linked together.

We are indebted to Dr. J. David and Dr. R. Schwartz for their critical review of this paper.

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