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Suppression of Antibody Synthesis to Dinitrophenylated Bovine Gamma Globulin in Contact-sensitized Guinea Pigs[1] (38868)

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Contact sensitization to the halogenated nitrobenzenes has long been used to establish the lack of interdependence between cellular and humoral immune responses (1). A recent demonstration to support this view emerges from the finding that sensitization to 2,4-dinitrochlorobenzene (DNCB) is induced as readily in guinea pigs rendered tolerant to the dinitrophenyl (DNP) determinant as in nontolerant controls (2). It has now been shown that DNCB also induces a humoral response in all guinea pigs sensitized to this simple chemical (3). We, therefore, sought to address the converse question, namely, does the induction of a delayed response to DNCB influence antibody production to DNP–protein conjugates. The data presented below show that antibody formation to dinitrophenylated bovine gamma globulin (DNP·BGG) may be specifically suppressed in DNCB-sensitive guinea pigs.

Materials and Methods. Sensitization of guinea pigs to DNCB. Male Hartley strain guinea pigs were sensitized by epicutaneous application of 1 mg of DNCB or 100 μg of DNCB in complete Freund's adjuvant (CFA) (Difco Labs., Detroit, MI) as described in (3).

Pretreatment with CFA alone. The injection material was prepared and administered as described in (3) for the injection of 100 μg DNCB in CFA, except for the replacement of the DNCB by 95% ethanol.

Immunization with DNP–BGG and human serum albumin (HSA). DNP–BGG conjugates were prepared by reacting bovine gamma globulin (Mann Research Labs., Rochester, NY) with 2,4 dinitrobenzene sulfonic acid (Eastman Kodak Co., Rochester, NY) at alkaline pH as described in (4). The degree of conjugation of the protein was assessed by absorbancy readings at 360 nm and dry-weight determinations.

A 20 mg/ml solution of HSA (Behring Diagnostics, Sumerville, NJ) was prepared in distilled water, spun at 20,000 rpm for 30 min, and standardized spectrophotometrically.

Appropriate dilutions of the antigens in 0.15 *M* NaCl were admixed with equal volumes of CFA so that each guinea pig received 100 μg of protein and 0.12 mg dry weight of mycobacteria in 0.5-ml vol injected in four nuchal area sites.

Collection of sera. All animals were bled 14 days after the injections indicated in the tables of DNP·BGG or HSA. The sera were collected and stored at −20°C.

Antibody assays. Serum anti-DNP and anti-HSA levels were estimated by the mABC assay (5) with the modifications described in (3). All sera were diluted 1:26 with PBS-E as in (3). Pooled sera from untreated animals similarly diluted, provided the baseline values for the assays.

Experimental design. To characterize the effects of contact sensitization with DNCB on the humoral response to the DNP determinant, guinea pigs were pretreated as outlined in Tables I through III and injected with DNP–BGG or with an unrelated antigen, HSA. A typical experiment comprised seven groups of 10–15 guinea pigs each. Group A animals were injected with DNP–BGG or HSA only and provide the control values for the response to these antigens. Groups B thru E were sensitized with DNCB before the injection of DNP–BGG or HSA. Sensitization to DNCB was accomplished in groups B and C by epicutaneous application and in groups D and E by injection into the toe-pads with CFA.

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TABLE I. THE EFFECT OF A PRIOR TREATMENT WITH DNCB ON THE ANTIBODY RESPONSE OF GUINEA PIGS TO 100 μg $DNP_{31}BGG$ IN CFA.

Group[a]	Pretreatment	Time of pretreatment day	Anti-DNP (μg N/ml serum) $\bar{m} \pm SE$[b]	Range	P[c]
A	None		143±9	67–234	
B	1 mg DNCB epicut.	−3	60±7	17–114	<0.001
C	1 mg DNCB epicut.	−7	67±6	38–116	<0.001
D	100 μg DNCB in CFA	−3	102±9	61–177	<0.01
E	100 μg DNCB in CFA	−7	113±9	49–195	<0.02
F	CFA only	−3	131±9	74–210	NS[d]
G	CFA only	−7	110±9	41–189	<0.02

[a] All guinea pigs were bled 14 days after the injection of 100 μg $DNP_{31}BGG$ in CFA. There were 15 animals in each group.

[b] Standard error of the mean.

[c] All values are compared with the mean for the controls in Group A.

[d] Not significant at the level of $P \geq 0.05$.

The animals in groups F and G received only CFA. These groups provided the control values for the effect of CFA on the subsequent antibody response. The induction of contact sensitivity in groups B thru E was confirmed by the skin-testing procedures described in (3) in groups maintained concurrently with the experimental animals.

Statistics. Within each experiment the antibody responses in groups E thru G were compared to that in group A by means of Student's *t* test.

Results. Immunosuppressive effect of DNCB on the response to $DNP_{31}BGG$. The data in Table I clearly indicate that serum anti-DNP levels are lower in animals sensitized with DNCB than in the controls. The suppression was more marked in the animals painted with DNCB than in those injected with the hapten. In the former, the mean anti-DNP values were 60 ± 7 and 67 ± 6 μg anti-DNP N per ml serum (Groups B and C), less than half the mean of 143 ± 9 μg N for the controls in Group A. In the guinea pigs injected with DNCB in CFA, significant suppression occurred when the hapten preceded the DNP–protein conjugate by 3 days (Group D), and to a lesser extent at the 7-day interval. The humoral response in animals injected with CFA only was lower for Group G but not for Group F guinea pigs.

Immunosuppressive effect of DNCB on the response to $DNP_{62}BGG$. The experiment summarized in Table II was performed to evaluate the immunosuppressive effect of DNCB on antibody formation to the more highly substituted conjugate, $DNP_{62}BGG$. DNCB administration also suppressed this response but the diminution was more transitory and lesser in extent than that observed with $DNP_{31}BGG$. A significant drop in the amount of serum anti-DNP levels was observed only in those guinea pigs painted with DNCB 3 days prior to the injection of $DNP_{62}BGG$ (Group B, Table II). It is also noteworthy that the anti-DNP levels in every group injected with $DNP_{62}BGG$ were higher than in the animals immunized with $DNP_{31}BGG$ (cf. Tables I and II).

Specificity of the immunosuppressive effect attributable to DNCB. The experiment summarized by the data in Table III was designed to evaluate the specificity of immunosuppression due to DNCB. Guinea pigs pretreated with DNCB or with CFA were challenged with HSA. As seen in Table III, antibody production to HSA attained equivalent levels in the controls, Group A, and in the guinea pigs which received DNCB epicutaneously (Groups B and C).

In contrast, the injection of CFA with or without admixture of DNCB, significantly diminished the synthesis of antibody to HSA (Groups D thru G, Table III). Indeed, the lowest response was observed in the guinea pigs injected only with CFA 1 week prior to immunization with HSA in CFA. The mean

TABLE II. THE EFFECT OF A PRIOR TREATMENT WITH DNCB ON THE ANTIBODY RESPONSE OF GUINEA PIGS TO 100 μg $DNP_{62}BGG$ IN CFA.

Group[a]	Pretreatment	Time of pretreatment day	Anti-DNP (μg N/ml serum) $\bar{m} \pm SE$[b]	Range	P[c]
A	None		180±24	100–360	
B	1 mg DNCB epicut.	−3	95±13	54–126	<0.01
C	1 mg DNCB epicut.	−7	129±15	72–217	NS[d]
D	100 μg DNCB in CFA	−3	189±38	50–425	NS
E	100 μg DNCB in CFA	−7	188±17	95–277	NS
F	CFA only	−3	155±21	93–295	NS
G	CFA only	−7	135±23	43–249	NS

[a] All guinea pigs were bled 14 days after the injection of 100 μg $DNP_{62}BGG$ in CFA. There were 10 animals in each group.

[b] Standard error of the mean.

[c] All values are compared with the mean for the controls in Group A.

[d] Not significant at the level of $P \geq 0.05$.

TABLE III. THE EFFECT OF A PRIOR TREATMENT WITH DNCB ON THE ANTIBODY RESPONSE OF GUINEA PIGS TO 100 μg HSA IN CFA.

Group[a]	Pretreatment	Time of pretreatment day	Anti-HSA (μg N/ml serum) $\bar{m} \pm SE$[b]	Range	P[c]
A	None		108±10	78–186	
B	1 mg DNCB epicut.	−3	90±7	60–107	NS[d]
C	1 mg DNCB epicut.	−7	122±7	93–168	NS
D	100 μg DNCB in CFA	−3	65±6	34–93	<0.01
E	100 μg DNCB in CFA	−7	70±9	34–124	<0.01
F	CFA only	−3	72±6	41–106	<0.01
G	CFA only	−7	49±5	34–79	<0.001

[a] All guinea pigs were bled 14 days after the injection of 100 μg HSA in CFA. There were 10 animals in each group.

[b] Standard error of the mean.

[c] All values are compared with the mean for the controls in Group A.

[d] Not significant.

anti-HSA N value for the sera of these animals was 49 ± 5 μg N per ml, or less than half the mean of 108 ± 10 observed in the controls.

Duration of the immunosuppressive effect due to DNCB. Ten guinea pigs were painted with 1 mg of DNCB, while 10 others were injected with 100 μg DNCB in CFA. A third group of 10 animals was set aside for control purposes. Seven and 14 days later the 20 immunized guinea pigs were skin-tested with a series of DNCB solutions. The total amount of DNCB used in the two skin test series was 148 μg (see ref. 3). Ten weeks after the initial sensitization with DNCB, all 30 guinea pigs were injected with 100 μg DNP_{31}-BGG in CFA and bled 14 days later. The mean serum antibody levels for each of the groups were: (1) Painted with 1 mg DNCB 75 ± 12 μg anti-DNP N per ml; (2) Injected with 100 μg DNCB in CFA 138 ± 10; (3) Controls 200 ± 20.

The P value for the means of Groups 1 vs 3 is <0.001 whereas the difference between the means of Groups 2 and 3 were not significant.

Discussion. The results described above demonstrate that a single administration of DNCB induced contact sensitivity and specifically suppressed humoral antibody for-

mation to DNP–BGG. The suppressive effect persisted for several weeks and varied with the mode of DNCB administration as well as with the immunogenic potency of the DNP–BGG conjugates used for inducing the humoral response.

As shown in Table I serum anti-DNP levels were significantly reduced in guinea pigs receiving epicutaneous DNCB and an injection of $DNP_{31}BGG$. The suppression was specific since antibody levels to HSA were unaffected by painting the guinea pig skin with DNCB (Table III). A lesser degree of suppression followed the injection of DNCB in CFA (Table I), possibly due to the adjuvant effect of CFA with respect to serum antibody formation (3) the route of administration or the small amount of DNCB (0.1 mg) used in these animals as compared to their counterparts painted with 1 mg of DNCB. The latter treatment was considerably less effective in animals challenged with $DNP_{62}BGG$ (Table II). These guinea pigs also mounted a more intense response (180 ± 24 μg anti-DNP N per ml) as compared with those immunized with DNP_{31}-BGG (143 ± 9 μg anti-DNP N per ml). This result is attributable to the higher epitope density of $DNP_{62}BGG$ as compared to $DNP_{31}BGG$ (6, 7) which annulled the tolerogenic effect of DNCB (cf. Tables I and II).

The suppression of the humoral response after the administration of DNCB that we have now documented cannot be related to the delayed skin response simultaneously induced by this compound. Dermal sensitivity is more intense in guinea pigs pretreated with the contactant in CFA than in those which receive a percutaneous application (3), yet the latter were more readily suppressed in terms of serum antibody levels. Furthermore it has been previously shown that nitrobenzenes administered by tolerogenic routes (8, 9) or conjugated to autologous or isologous proteins (10–12) so that they do not elicit contact sensitization, are also suppressors of the response to the DNP determinant. These considerations, therefore, denote a clear dissociation between the cellular and humoral responses. The degree of skin sensitivity does not reflect the animal's capacity to produce a humoral response, nor does the extent of the former influence serum antibody levels.

Unpublished experiments in this laboratory suggest that lymphoid cell receptors specific for the DNP determinant may be blocked by prior administration of DNCB and thereby contribute to its tolerogenicity. The data in this report do not provide further information on the possible mechanism of tolerance induction by DNCB. They do indicate, however, that a single experience with this hapten evokes a multiple response which includes the development of contact sensitivity and partial tolerance, as well as low levels of antibody synthesis (3). The findings do suggest that the coexistence of cellular and humoral immune responses although induced through different pathways may exert a mutually modulating effect, as has been proposed in studies of tumor immunity and allograft rejection (13).

The data in Table III indicate that the injection of CFA within 1 wk before inoculation of HSA in CFA exerts a suppressive effect on the anti-HSA response. Similar results have been reported with this and other antigens and possible mechanisms for this effect have been suggested (14, 15). The suppressive effect of CFA was also noticeable with $DNP_{31}BGG$ but not with the more potent immunogen, $DNP_{62}BGG$.

Summary. The anti-DNP response to DNP-BGG is substantially suppressed in guinea pigs sensitized to DNCB. The degree of antibody suppression varies with the mode of skin sensitization and with the degree of conjugation of the challenging immunogen. Suppression of the anti-HSA response was also induced by the prior injection of CFA.

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