HETEROLOGOUS CARRIERS IN THE ANAMNESTIC ANTIHAPTEN RESPONSE*

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To obtain anamnestic antibody synthesis to a second antigenic stimulus the second antigen must share some immunogenic determinant with the primary sensitizing material. Generally the secondary response takes place only to the common determinants (1–3). Occasional animals have been reported to undergo a secondary antibody response to antigenic determinants specific only to the first antigen when injected with a cross-reacting antigen as the secondary stimulus (1, 4). While the antibodies produced appeared to be specific for the first antigen based on absorption of the secondary sera with the second antigen, the complexity of the antigens (albumins) has limited the interpretation of these results. Albumins are multispecific antigens (5, 6) and the extent to which hidden determinants of one species might cross-react with surface determinants on a cross-reacting species has not been investigated.

An artificial haptenic group can represent a significant portion of a specific determinant. Subtle alterations in the spacial configuration of haptens cause corresponding alterations in the elicited antihapten antibodies and are distinguishable by them (7). However, in the past most experiments designed to induce a specific anamnestic antihapten circulating antibody response independent of the carrier molecules have been unsuccessful (8–12). An exception was an experiment by Gold and Benedict (13) in which small increases in anti-*p*-aminobenzoic acid antibody levels were detected by hemagglutination following administration of uncoupled hapten. The more common negative result led to the general conclusion expressed by Ovary and Benacerraf (10) that the antihapten-anamnestic response is controlled in part by the "immunological backbone" of the carrier and that for this reason the original carrier is required

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to stimulate a secondary antihapten response. Recently, however, several reports indicated that this need not be the case and that it is possible for an unrelated carrier protein to serve as a secondary hapten carrier and that such a combination can induce an anamnestic antihapten response which in most but not all cases was of limited intensity (14–18).

The purpose of this paper is to describe fully experiments presented in a preliminary report (15) concerning the anamnestic production of anti-TNP (trinitrophenyl) precipitins in rabbits primed with trinitrophenyl-hemocyanin (TNP-KLH) and recalled with TNP–bovine plasma albumin (TNP-BSA) or TNP–bovine gamma globulin (TNP-BGG) at intervals of up to 11 months after priming. The resultant antihapten responses were equal in magnitude to those recalled by the TNP–homologous carrier conjugate and independent of the measurable response to sensitization.

Materials and Methods

Proteins.—Hemocyanin (KLH) was obtained from the keyhole limpet *Megathura crenulata* by the method of Campbell et al. (19). Crystalline bovine plasma albumin (BSA) and bovine gamma globulin (BGG) were purchased from Armour Pharmaceutical Company, Kankakee, Ill. Rabbit serum albumin (RSA) was precipitated by acetic acid from pooled normal rabbit serum after removal of the globulins by precipitation with half-saturated ammonium sulfate. Precipitated albumin was dissolved and dialyzed against distilled water and concentrated by lyophilization. The protein was purified by preparative starch block electrophoresis (19). The material was dialyzed free of salt with distilled water, clarified by low speed centrifugation, and lyophilized. It was judged pure by immunoelectrophoresis.

Preparation of Conjugates.—TNP-KLH was prepared as described previously (20). Mole ratios of TNP to KLH ranged from $TNP_{520}KLH$ to $TNP_{1280}KLH$ as indicated in the text.

TNP-BSA was prepared as in reference 20 except that the coupling reaction between BSA and trinitrobenzene sulfonic acid (TNBS), the reactive form of the hapten, was stopped by the addition of 10 moles of glycylglycine per mole of hapten in the reaction mixture (21). The conjugated protein was precipitated by the addition of cold 1 M acetic acid and centrifuged. Borate buffer pH 8.6 (19) dissolved the precipitated conjugate. After centrifugation the clarified protein was passed through a Sephadex G-50 column equilibrated in borate-saline (19) to remove free TNBS and TNP-glycylglycine. The cycle of precipitation and gel filtration was repeated once or twice more until the optical density ratio at 350 mμ/280 mμ of the conjugate in borate-saline was the same after two successive column passages. Mole ratios of TNP to BSA prepared in this manner ranged from $TNP_{11}BSA$ to $TNP_{14}BSA$.

TNP-RSA was prepared similarly except that the coupling reaction was carried out in borate buffer pH 8.1 for 4 hr. The mole ratio of TNP to RSA was TNP_{22}/70,000 mol wt RSA.

TNP-BGG was prepared by reacting 0.19 g TNBS with 0.75 g BGG in 15 ml 0.1 M phosphate buffer pH 7.9 at 30°C in the dark. The reaction was stopped by addition of glycylglycine as above. During coupling the protein precipitated. It was collected by centrifugation and dissolved by bringing the pH to 7.9 with 0.5 N NaOH. The conjugate was passed through Sephadex G-50 equilibrated in 0.1 M phosphate-buffered saline pH 7.9. The conjugated protein again precipitated on standing in the cold and was used as a suspension for injection. There were approximately 13 moles of TNP/1.6×10^5 mol wt BGG.

In order to eliminate the possibility of contamination of one protein preparation by another a new Sephadex gel was used for each procedure. A single gel was never used for more than a single kind of protein.

Spectrophotometric determinations of hapten per protein were performed as in reference 20.

ε-mono-TNP-L-lysine was synthesized by a modification of Okuyama and Satake's method (22) using 2.4 g of L-lysine:HCl and 9.6 g of picryl chloride (1 chloro, 2, 4, 6 trinitrobenzene) obtained from Matheson, Coleman and Bell (Cincinnati, Ohio). After washing with benzene the product solution was adjusted to pH 3 with 6 N NaOH and the resulting precipitate discarded. The solution was adjusted to pH 6 with 1 N NaOH and allowed to stand for several hours in the cold whereupon the product crystallized. Recrystallization was repeated twice at pH 6. The final product crystallized at pH 3. It was washed with absolute ethanol and ether and dried over P_2O_5, mp 198–199°C. Elemental analysis,[1] calculated for the hydrochloride C, 35.00; H, 4.41; N, 17.01; Cl, 8.61; O, 35.00. Found C, 35.59; H, 4.46; N, 17.47; Cl, 8.61; O, 35.24.

Antisera.—Adult, male, New Zealand rabbits were used. All injections were intravenous without adjuvants. Antigen concentrations used for injection ranged from 10 to 15 mg protein per milliliter saline. The total dose per injection was 15 mg protein per rabbit in all but the first experiment in which four rabbits received 25 mg protein each in the initial injection. Sensitization in all but one experiment consisted of two injections administered 18 days apart. The last experiment involved a single primary injection with no boosters prior to challenge. Animals were bled from the ear at various intervals. Sera were stored in a freezer until tested.

Quantitative Precipitin Analysis.—Duplicate tests were performed as described previously (20). Antibody to KLH was determined using native KLH as precipitating antigen at pH 6.9. The first two experiments employed TNP-BSA as precipitating antigen for measuring antihapten antibody. Although control animals do not produce measurable amounts of anti-TNP-BSA when given 15 mg of TNP-BSA the possibility exists that a small portion of the antibody labeled anti-TNP in Fig. 1 is actually coprecipitated anti-TNP-BSA. Subsequent experiments employed TNP-RSA as precipitating antigen for determining anti-TNP antibody recalled with TNP-BSA.

RESULTS

Six rabbits were challenged 120 days after the second of two sensitizing injections of TNP-KLH. Three animals were challenged with 15 mg TNP_{665} KLH and three with 15 mg TNP_{12}BSA. Two rabbits in each group produced measurable amounts of anti-TNP (Fig. 1). All six produced precipitins detectable by ring test. Only the three rabbits challenged with TNP-KLH showed an elevation in anti-KLH titer. In this and in all subsequent experiments prechallenge injection bleedings revealed low levels of circulating anti-KLH precipitins persisting in nearly all of the rabbits as a result of previous exposure to TNP-KLH. This low level was not measurable and is indicated by the points below 100 μg in Fig. 1. Anti-TNP precipitins were not detected in any rabbit at time of challenge. The data indicate an anamnestic antihapten response recalled by the hapten–heterologous carrier complex because there was no equivalent rise in anti-KLH or anti-BSA (not shown) antibody and because control rabbits do not respond measurably to a single intravenous injection of TNP-BSA.

All ring test–positive sera were tested for ability to flocculate by mixing equal volumes of serum and antigen and incubating at 37°C for 2 hr. Twofold dilu-

[1] Elemental analysis performed by Elek Microanalytical Laboratories, Torrance, Calif.

tions of antigen from 160 μg N/ml to 2.5 μg N/ml were used (19). The statement that certain sera did not contain measurable amounts of antibody indicates that they did not flocculate in this preliminary test. None of the control animals used in this investigation produced measurable amounts of antibody after a single intravenous injection of either TNP-BSA or TNP-BGG.

In a second experiment only 37 days elapsed between the second sensitizing

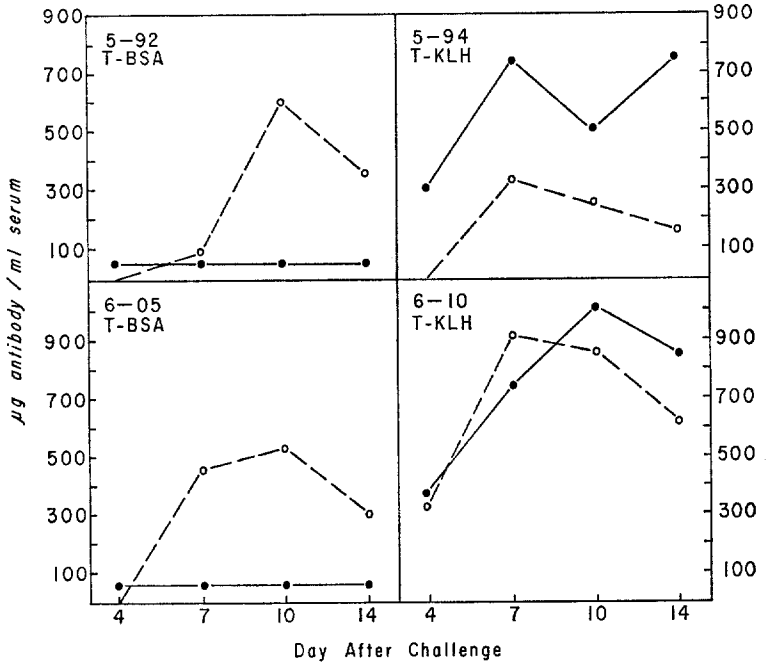

FIG. 1. Recall of anti-TNP (○---○) and anti-KLH (●——●) antibody by challenge 120 days after second sensitizing injection. Rabbits 5-92 and 5-94 were sensitized with 15 mg each and rabbits 6-05 and 6-10 with 25 mg each of $TNP_{1081}KLH$. Each of the four was reinjected 18 days later with 15 mg of $TNP_{569}KLH$. The challenge injection consisted of 15 mg of either $TNP_{665}KLH$ or $TNP_{12}BSA$ as indicated in the figure.

injection of TNP-KLH and challenge with 15 mg $TNP_{13}BGG$. Four rabbits were so injected along with 10 normal control animals. While none of the controls showed antibody specific for the hapten, two of the four test rabbits did (Table I). A single control rabbit produced detectable precipitins to the heterologous carrier protein BGG while three showed a response to the hapten-carrier complex TNP-BGG (Table III). As in the first experiment there was no corresponding rise in anti-KLH titer (not shown). The two recall rabbits responding to the hapten–heterologous carrier produced significant amounts of antihapten antibody (Table II). Although there was no detectable antibody to BSA

the possibility was entertained that a small portion of the antibody to TNP as measured by TNP-BSA was actually to the hapten-protein complex TNP-BSA as a result of the contamination by BSA of commercially prepared BGG. To test this possibility hapten inhibition by the method of Pauling, Pressman, and Grossberg (23) was carried out on rabbit 7-59 7-day recall serum. ϵ-TNP-lysine was employed as inhibiting hapten with antigen and antibody at equivalence (Fig. 2). The shift in slope of the dashed line near complete inhibition suggests a heterogeneity which may reflect antibody directed against determinants

TABLE I

Ring Tests on Sera from TNP-KLH-Sensitized Rabbits after Recall with TNP-Heterologous Carrier Conjugates*

Recall antigen	Days to recall	Day after recall	Ring test antigen‡					
			T-BSA	T-BGG	T-RSA	BSA	BGG	RSA
$TNP_{13}BGG$	37	7	2/4§	3/4		0/4	0/4	
		10	2/4	3/4		0/4	0/4	
		14	2/4	3/4		0/4	0/4	
$TNP_{14}BSA$	77	4	1/5	0/5		0/5		0/5
		7	4/5	3/5	3/5	2/5		0/5
		11	5/5	3/5	3/5	2/5		0/5

* TNP-BGG group sensitized with two injections of 15 mg $TNP_{518}KLH$ administered 18 days apart. TNP-BSA group sensitized as above with $TNP_{784}KLH$. Recall injections 15 mg each.

‡ Antigen concentrations for ring tests 10 μg N/ml.

§ No. positive/No. tested. The lowest concentration of anti-TNP detectable by ring test was 25 μg/ml as determined with dilutions of antisera of known antibody content.

other than the hapten-lysine, perhaps TNP-N-terminal amino acid or hapten-BSA complex. TNP-lysine did not inhibit precipitation of BSA-rabbit anti-BSA (not shown).

A similar experiment was carried out after 77 days had elapsed between sensitization and heterologous recall with TNP-BSA. No response was seen in the control group until the 11th day after injection when precipitins to TNP-BSA were detected in two rabbits. In contrast all five test animals showed antibodies to the recall antigen and in three of these there was antibody specific for the hapten as shown by reactivity wth TNP-RSA and TNP-BGG (Table I). The sera of the heterologously recalled rabbits contained 0.5–1.2 mg anti-TNP by the 7th day after recall (Table II). As in other experiments there were no detectable anti-TNP precipitins in these rabbits just prior to challenge and there was no increase in anti-KLH after challenge. The specificity of the anti-TNP produced by rabbits 8-05 and 8-14 7 days after challenge was tested by

precipitation inhibition by ε-TNP-lysine at antigen-antibody equivalence (Fig. 2). The heterogeneity reflected by the slope of hapten inhibition in the TNP-BGG recall serum (dashed line) is absent in these sera. This result tends to reinforce the interpretation that a small portion of the apparent anti-TNP recalled by TNP-BGG as tested for with TNP-BSA actually was directed toward some determinant other than TNP-lysine. Specificity of the recalled antibody as measured by TNP-RSA is clearly antihapten.

Two additional experiments of this type were performed. The interval between sensitization and challenge was 3 months in one experiment and 11 months in the second. One of the groups received one sensitizing injection rather than two. The response to sensitizing injections was evaluated quantitatively

TABLE II

Antihapten Precipitin Titers in Sera of TNP-KLH–Sensitized Rabbits after Challenge with TNP–Heterologous Carrier Conjugates*

Recall antigen	Days to recall	Rabbit No.	‡Micrograms anti-TNP/ml serum	
			Day 7	Day 10–11
$TNP_{13}BGG$	37	7-59	675	519
		7-62	200	169
$TNP_{14}BSA$	77	8-05	513	400
		8-10	1275	675
		8-14	500	419

* As in Table I.

‡ Quantitative precipitin titers. TNP-BSA was precipitant for TNP-BGG–recalled sera. TNP-RSA was precipitant for TNP-BSA–recalled sera.

in order to see whether primary responsiveness to hapten had any effect on subsequent ability to respond to heterologous recall (Table IV).

Reducing to one-half the total antigen administered at sensitization and limiting it to a single primary injection did not affect recall of antihapten adversely. Extending the time between sensitization and challenge to 11 months did not affect the outcome adversely either. Whether or not a rabbit responded well to TNP after sensitization with TNP-KLH had no bearing on its response to challenge with TNP-BSA. Rabbits 8-36, 8-60, and 8-61 responded poorly if at all to sensitization yet were good anti-TNP producers on recall. On the contrary, rabbits 8-37, 8-39, and 8-43 responded well to sensitization but responded minimally or not at all to recall.

The responses of normal animals injected with the same TNP-BSA preparations used in the above experiments were minimal and not measurable by quantitative precipitation (Table III). None of the rabbits showed any reactiv-

ity with TNP-RSA and only 6/16 showed reactivity with the sensitizing antigen TNP-BSA as detected on the 10th or 11th day.

DISCUSSION

These results demonstrate an anamnestic antihapten precipitin response to hapten conjugated to a heterologous carrier. The amount of antihapten anti-

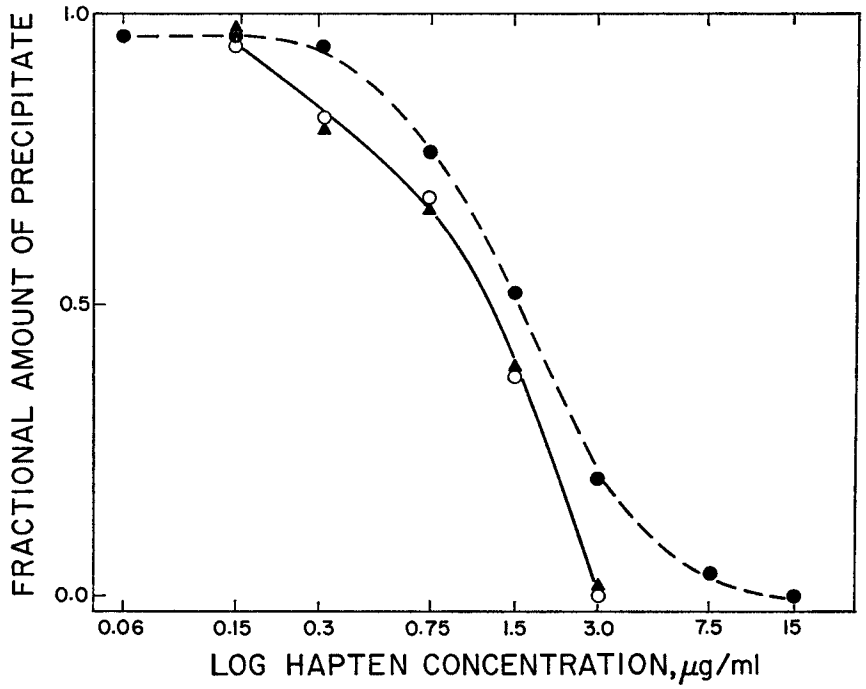

FIG. 2. Hapten inhibition of precipitation of recalled anti-TNP sera. Inhibition by ε-mono-TNP-L-lysine. ●—●, rabbit 7-59 challenged with 15 mg TNP-BGG 37 days after sensitization. Anti-TNP precipitated with TNP-BSA. ○——○, rabbit 8-05, and ▲——▲, rabbit 8-14 challenged with 15 mg TNP-BSA 77 days after sensitization. Anti-TNP precipitated with TNP-RSA.

Antisera were obtained 7 days after challenge. Inhibition was performed with antigen and antibody at equivalence.

Hapten similarly added to rabbit anti-BSA-BSA mixtures did not inhibit precipitation (not shown).

body so produced is comparable with that induced by the hapten–homologous carrier conjugate as described previously (20) and as shown here (Fig. 1).

Absence of cross-reaction between KLH and BSA as judged by failure of these proteins to cross-stimulate in vivo immunological processes has been shown (24, 25). During this investigation additional tests were made between

high titered anti-KLH or anti-BSA sera and the haptenated heterologous proteins. As tested by passive cutaneous anaphylaxis and gel diffusion there was no cross-reaction. Nor was there an increase in anti-KLH titer after recall with either TNP-BSA or TNP-BGG.

Carrier alone was not able to recall the antihapten response. 17 rabbits primed with TNP-KLH were challenged with KLH between 105 and 126 days after sensitization. All failed to produce detectable antihapten antibodies.

TABLE III

Responses of Normal Unsensitized Rabbits to Recall Doses of TNP–Heterologous Carrier Conjugates

Antigen*	Day after injection	Ring test antigen‡					
		T-BSA	T-BGG	T-RSA	BSA	RSA	BGG
$TNP_{13}BGG$	7	0/10§	3/10		0/10		1/10
	10	0/10	3/10		0/10		1/10
	14	0/10	3/10		0/10		1/10
TNP-BSA‖	4	0/11	0/11	0/11	0/11	0/11	
	7	0/16	0/11	0/16	0/16	0/11	
	10–11	6/16	0/11	0/16	1/16	0/11	
	14	4/11	0/6	0/11	0/11	0/6	

* Single intravenous injection, 15 mg/rabbit.

‡ As in Table I.

§ As in Table I.

‖ Three different preparations were used, conforming to those used as recall antigens. $TNP_{14}BSA$ and $TNP_{11}BSA$ each administered to five control animals. $TNP_{13}BSA$ administered to six control animals.

Although early experiments (26–28) indicated that immunologic recognition of antigenic regions of haptenated proteins included more of the carrier in delayed than in immediate hypersensitivity, recent experiments (29–32) have narrowed this distinction. Leskowitz et al. (30) showed that delayed but not immediate hypersensitivity reactions were elicited with arsanilate-protein in animals sensitized with arsanilate-*N*-acetyl-tyrosine indicating that a very small structural region can sensitize to delayed hypersensitivity. Schlossman and Levine (32) suggested that the size of the structural area surrounding the hapten as measured by delayed hypersensitivity reflects the functional region of hapten immunogenicity based on the ability of α-dinitrophenyl-hepta-L-lysine to both immunize and to elicit delayed hypersensitivity, functions which the hexapeptide lacked. Presumably the size of the region would vary with the materials employed. Whether the ultimate size of such an immunogenic region would ever be so narrow as to encompass the hapten alone is not yet clear. This is suggested, however, by the successful elicitation of delayed hypersensitivity by arsanilate-salmine in animals sensitized to arsanilate-polytyrosine (29). Salmine contains no tyrosine in-

dicating that the immunogenic haptenic determinant did not include specifically the associated amino acid.

The experiments reported here show that immunologically recognizable portions of the carrier "backbone" are not required in the homologous hapten–heterologous carrier recall of circulating antihapten antibody. While it is possible that submolecular regions of similarity occur in KLH and BSA and BGG these are beyond detection by present immunologic methods. Possibly the hapten singles out such submolecular regions. Unlike the chloride form of TNP, picryl chloride (which also attacks phenolic hydroxy and imidazole imino groups), the sulfonic acid form of TNP as used here conjugates to primary amino groups selectively (22). Haptenation by this method results in a more uniform product in which lysine accounts for nearly all of the conjugated amino acids except for the relatively small proportion of available N-terminal groups. While the immunogenic region may be larger than the hapten and adjacent amino acid or may include regions linearly distant due to spatial foldings of the carrier, a certain degree of homogeneity is achieved by this haptenation procedure. Whether reduced heterogeneity in the site of hapten attachment affects success or failure of heterologous recall is not known. If submolecular environment plays a role in hapten immunogenicity, multiple environments on the sensitizing carrier could provide a better chance for finding some homologous environments when hapten is attached to other proteins. From a selective point of view, however, homogeneity of hapten attachment would result presumably in a reduced spectrum of cells responding to sensitization. In this sense proliferation of a narrower class of antihapten-specific cells might help to magnify the recall response when the hapten is presented on a carrier providing the proper environment. It should be noted that thus far attempts to recall anti-TNP with mono-TNP-bovine-insulin have failed.[2] In this case insulin provided a very homogeneous environment with but a single lysine located on the B chain accounting for approximately 95% of the hapten. The remaining hapten was found on the N-terminal amino acids glycine and phenylalanine. Since insulin is a weak immunogen the reason for failure cannot be attributed directly to the hapten-carrier structural relationship. Other proteins or peptides of known structure and greater immunogenicity may offer a better means of testing this aspect of heterologous recall.

While all of the animals responded to heterologous recall when challenged 11 months after sensitization it was possible also to obtain significant recall after only 37 days had elapsed between sensitization and challenge. Although the groups were small the proportion of positive responders (approximately 50%) was the same at challenge intervals lying between 37 days and 11 months. Therefore, while a very lengthy interval may enhance the ability of some ani-

[2] Rittenberg, M. B. Unpublished observations.

mals to respond it is not an absolute requirement as was suggested by similar experiments employing pneumococcus and pneumococcal polysaccharide as sensitizing immunogen and challenging hapten respectively (18). These variations in time of successful recall may reflect differences in the fundamental immunogenicities of the materials employed as well as differences in size of individual cell populations capable of responding to a particular stimulus. They

TABLE IV

Comparison of Response to Sensitization by TNP-KLH and Subsequent Ability to Respond to Heterologous Recall by TNP-BSA

Rabbit No.	Sensitizing injections		Microgram anti-TNP*/ml serum								
			Day after last sensitizing injection					Days to recall‡	Day after recall injection		
			4	6–8	10	12	14		6–8	10	14
8-36	15 mg	15 mg	0§	0					444	306	219
8-37	T_{837}KLH	T_{960}KLH	294	556					0	0	0
8-38			294	625‖				92	175	147	+
8-39			+	275					0	+	+
8-42			275	275‖					638‖	663	531
8-43			481	463					0	0	+
8-57	15 mg	None		0	150	112	150		806	606	456
8-58	T_{1022}KLH			0	269	244	306		188	131	
8-59				+	+	144	119	333	225	147	+
8-60				0	+	+	131		1225	394	300
8-61				0	0	0	0		394	256	+

* Anti-TNP as measured by quantitative precipitation using TNP-BSA as precipitant before recall; TNP-RSA as precipitant after recall.

‡ 8-36 through 8-43 recalled with 15 mg TNP_{13}BSA. 8-57 through 8-61 recalled with TNP_{11}BSA.

§ 0, antibody not detectable by ring test. +, antibody detectable by ring test but amount insufficient to measure (see text).

‖ Antibody values after absorption of complement and nonspecific factors by treatment with washed heterologous antigen–antibody complexes (20).

may also reflect the suppressive effects of residual antihapten antibody (33). Although these experiments were carried out after anti-TNP precipitins had disappeared, ring testing is too insensitive a method to insure that all animals had the same residual level of circulating anti-TNP at the time of recall.

These studies were undertaken initially because TNP-KLH had proved to be a potent immunogen which does not require adjuvant in order to elicit early precipitating antihapten antibody (20). It was felt that previous failures to demonstrate anamnestic antihapten responses independent of the carrier (8–12)

may have been due to weaker immunogenic stimuli and that TNP-KLH could overcome this deficiency. The data in Table IV which show no correlation between anti-TNP response after primary sensitization and subsequent ability to be challenged successfully by TNP–heterologous carrier conjugate indicate that potent immunogenicity of primary sensitization does not guarantee successful recall. Perhaps circulating antibody levels do not reflect the extent of sensitization. Although antihapten titer presumably parallels the size of the cell population producing specific antihapten antibody, the correlation may be too gross to provide a satisfactory index of sensitization, particularly if only certain subpopulations of cells producing antihapten antibody will respond when the challenging hapten is on a heterologous carrier. If the latter were the case however, the heterologously recalled response would not be expected to equal the homologous response quantitatively yet it did in many animals. The response of some cells to sensitization may be proliferative but nonantibody producing until a second antigenic contact occurs. Whereas others may respond directly to sensitization and produce antibody or continue proliferation to a stage where they respond to persisting primary antigen (34).

These experiments differed from others mainly in the use of a primary immunogen, TNP-KLH, which did not require adjuvant to elicit a primary antihapten response. Elimination of adjuvants made possible recall experiments at a time when antihapten antibodies were no longer detectable. In the other reported successful experiment in which recall responses were determined quantitatively antihapten titers only increased to twice the prerecall level and adjuvant was used during sensitization (18). Suppression by persisting antihapten antibody could have been the reason for failure or low level responses in that experiment although it did not suppress control animals injected with homologous conjugates. Presumably if persisting antibody were suppressing, then the greater the response to sensitization the greater would be the chance for suppression; the frequency of successful heterologous recall should be inversely proportional to primary response. The data in Table IV do not allow such a conclusion. There is no distinct correlation in either direction.

These experiments show that large immunologically identifiable regions of the primary carrier molecule are not required to elicit a sizeable secondary antihapten precipitin response. Immunologically unrelated proteins may serve as hapten carriers for heterologous recall of antihapten. The extent to which regions of submolecular homology between primary and secondary carrier contribute to antihapten recall remains to be determined.

SUMMARY

Anamnestic antihapten responses were obtained to trinitrophenyl (TNP) when rabbits sensitized to trinitrophenyl-hemocyanin (TNP-KLH) were challenged with TNP–heterologous protein conjugates. Hapten–heterologous

carrier conjugates elicited antihapten titers similar in magnitude to those elicited by the homologous carrier conjugate. Hapten–heterologous carrier recall of antihapten was successful as early as 37 days and as late as 11 months after sensitization. There was no correlation between anti-TNP–precipitating antibody titer after sensitization and the ability to respond to challenge by hapten–heterologous carrier. The results are discussed in terms of immunogenicity of sensitization, suppressive effects of persisting postsensitization antibody, and submolecular haptenic environment as factors possibly affecting the heterologous recall process.

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