Brief Definitive Report

Ir GENE CONTROLLED CARRIER EFFECTS IN THE INDUCTION AND ELICITATION OF HAPTEN-SPECIFIC DELAYED-TYPE HYPERSENSITIVITY RESPONSES*

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We have recently demonstrated that genes in the V_H linkage group control the fine specificity of the delayed-type hypersensitivity (DTH) response to nitrophenyl acetyl (NP) (1). It was shown that transfer of reactivity to NP was both T-cell dependent and required I-A homology between the donor and recipient animals (1). The studies described below were undertaken to further clarify the specificity of the responding T cell, as well as the specificity of other cells which could be involved in the DTH pathway.

In our earlier studies, cyclophosphamide-pretreated mice were primed with NP-bovine gamma globulin (NP-BGG), and NP-specific DTH responses were elicited by challenge with NP on a heterologous carrier, NP-bovine serum albumin (NP-BSA) (1). Because of considerable accumulated evidence from this laboratory of carrier effects in DTH reactivity (2–3),[1] the role of the carrier in the various phases of the NP-specific DTH response was explored. To analyze the carrier requirements in this response more definitively, we chose to use carriers, the immune response to which are under H-linked Ir gene control. The use of such carriers was motivated by the fact that for an Ir-restricted antigen, such as poly-(L-glu^{56}-L-lys^{35}-L-phe^{9}) (GLØ), strains of mice of the appropriate H-2 haplotype do not manifest detectable immune responses or carrier specific helper function (4, 5). Thus, by examining the effect of this antigen on the various phases of the hapten-specific DTH response in responder and nonresponder strains of mice, we were able to gain insight into the role of the carrier moiety in the induction and elicitation of NP-specific DTH responses.

Materials and Methods

Animals. All mice were purchased from The Jackson Laboratory, Bar Harbor, Maine, or were bred in the animal facilities of Harvard Medical School. Mice were used at 2–12 mo of age, and were maintained on laboratory chow and acidified, chlorinated water ad libitum.

Antigens. The linear terpolymer GLØ, lot GLP2, average mol wt 45,000, was prepared by Miles-Yeda, Ltd., Rehovot, Israel. The random linear copolymer, poly-(L-glu^{60}-L-lys^{40}), GL, lot 33, was purchased from Pilot Chemicals, Boston, Mass. BSA and BGG were purchased from Sigma Chemical Co., St. Louis, Mo. The preparation of NP-conjugated proteins has been previously described (1). The molar conjugation ratio of haptenic group used in this work were NP_{17}-BGG, NP_{11}-BSA, NP_{14}-GLØ, and NP_{16}-GL.

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[1] Kipps, T. J., J. Z. Weinberger, B. Benacerraf, and M. E. Dorf. Comparison of T and B cell reactivity to antigens under Ir gene control. Manuscript submitted for publication.

Immunization and Challenge. All animals described below, were pretreated with 20 mg/kg cyclophosphamide in 0.2 ml phosphate-buffered saline (PBS), injected intraperitoneally, 3 d before immunization. The antigens were emulsified, in complete Freund's adjuvant (CFA) (Difco Laboratories, Detroit, Mich.) containing 0.5 mg/ml *Mycobacterium butyricum*, so that the antigen concentration was 500 μg/ml of CFA emulsion. A total of 0.2 ml of emulsion was injected subcutaneously bilaterally divided between two sites on the dorsal flanks.

7 d after immunization, the mice were challenged. The DTH response was elicited by injecting, into the left footpad, 25 μl of a solution containing 1 mg/ml of the challenge antigen in PBS. The amount of footpad swelling was measured, using an engineer's micrometer, 24 h after challenge. Swelling was determined as the difference, in units of 10^{-4} in, between the left footpad thickness and the right footpad thickness. All animals were measured by two independent observers, whose measurements were then averaged.

Statistical Analysis. Analysis of average differences of footpad swelling obtained was performed with the Wang Programmable calculator. The arithmetic means and SEM were expressed, as well as the relevant P value, obtained with the two-tailed Student's t test. In this paper, any result referred to as significant, had a P value of $\ll 0.01$, the P value of nonsignificance was $P > 0.05$, unless otherwise indicated.

Results

Carrier Requirements for the Induction of NP-specific DTH. Mice of various strains were injected with 20 mg/kg cyclophosphamide. 3 d later, the mice were primed with 100 μg NP-BGG, NP-GLØ, or NP-GL in CFA, subcutaneously. Animals in the negative control groups received PBS in CFA. 7 d after priming, all animals were challenged with 25 μl of a 1 mg/ml PBS solution of NP-BSA. 24 h after challenge, the amount of footpad swelling was determined as the difference between the injected and uninjected footpad. The amount of footpad swelling was an indication of NP-specific priming by the various compounds.

As seen in Table I, all mouse strains examined mount a significant NP-specific response when primed with NP-BGG compared to the background responses observed in control mice which were primed with CFA alone. The ability of NP-GL to prime for an NP-specific response was then examined. GL is unable to induce an antibody response in all strains of mice thus far examined (5, 6). Similarly, NP-GL was unable to prime for an NP-specific response in the four strains examined. This suggested that a nonimmunogenic carrier could not be used for priming the DTH response.

The situation for a carrier which is immunogenic in only some strains was then

TABLE I

*Carrier-mediated Genetic Control of Induction of NP-specific DTH by NP-GLϕ and NP-GL**

Strain	H-2 formulae (KABJECSGD)	GLϕ response status	NP DTH response			
			NP-BGG primed	NP-GL primed	NP-GLϕ primed	CFA primed
BALB/c	ddddddddd	+	43.8 ± 1.8‡	13.2 ± 2.0	NT	11.5 ± 3.1
B10.D2	ddddddddd	+	49.2 ± 3.5‡	NT	52.4 ± 4.3‡	17.4 ± 1.8
B10.S(9R)	ss?kkdddd	+	42.8 ± 6.1‡	NT	43.8 ± 2.3‡	9.4 ± 2.2
B10.PL	uuuuuuuuu	+	36.6 ± 2.7‡	12.3 ± 3.4	53.1 ± 5.7‡	11.8 ± 3.9
C57BL/6	bbbbbbbbb	−	47.2 ± 3.7‡	18.6 ± 17.4	15.7 ± 1.8	17.2 ± 6.7
B10.GD	ddd?bbbbb	−	34.4 ± 1.9‡	11.3 ± 4.4	15.5 ± 5.0	6.7 ± 2.1
B10.S	sssssssss	−	44.1 ± 4.9‡	NT	2.4 ± 1.5	3.0 ± 2.8

NT, not tested.

* Groups of mice were cyclophosphamide pretreated with 20 mg/kg cyclophosphamide. 3 d later, groups of five mice each were primed with 100 μg of the indicated antigens in 0.2 ml of CFA. 7 d after priming, mice were challenged with 25 μl of a 1 mg/ml solution of NP-BSA in PBS. Footpad swelling was measured 24 h after challenge, in units of 10^{-4} in. The arithmetic means ± SE are presented.

‡ $P < 0.01$ as compared with CFA-primed negative control.

TABLE II
*Requirements for Elicitation of NP-specific DTH**

Strain	Priming antigen	DTH challenge		
		NP-BSA	NP-GLϕ	NP-GL
C57BL/6	NP-BGG	41.6 ± 4.6‡	43.4 ± 7.1‡	68.4 ± 7.1‡
	CFA	17.4 ± 2.3	10.0 ± 3.5	8.1 ± 3.1
BALB/c	NP-BGG	43.8 ± 1.8‡	35.2 ± 2.2‡	42.6 ± 1.6‡
	CFA	11.5 ± 3.1	10.2 ± 4.1	9.3 ± 2.2
B10.PL	NP-BGG	40.9 ± 3.2‡	43.3 ± 6.2‡	50.1 ± 4.9‡
	CFA	6.3 ± 2.0	7.9 ± 2.8	7.9 ± 3.98
B10.GD	NP-BGG	34.4 ± 1.9‡	38.1 ± 1.3‡	49.2 ± 13.8‡
	CFA	3.1 ± 1.5	7.9 ± 4.3	4.7 ± 2.3
B10.S	NP-BGG	47.2 ± 8.3‡	44.3 ± 5.2‡	33.5 ± 0.7‡
	CFA	5.9 ± 1.1	10.2 ± 3.0	5.5 ± 0.9

* Cyclophosphamide pretreated mice were primed with 100 μg of NP-BGG in CFA, or with CFA alone. 7 d later, groups of five mice each were challenged with 25 μg of the indicated compound in 25 μl of PBS. 24 h later, the net footpad swelling was measured in units of 10^{-4} in. The arithmetic means ± SE are presented.
‡ $P < 0.01$ as compared with CFA primed responses.

examined. GLØ is a random linear terpolymer, the response to which is under Ir gene control. When various strains of mice were primed with NP-GLØ, not all of the strains demonstrated an ability to prime for NP-specific DTH responses (Table I). Furthermore, the data shows a complete correlation between the ability of NP-GLØ to prime for NP-specific DTH responses and the cellular and humoral responder status to GLØ in the mouse strains examined. This data therefore indicates that the ability to prime for NP-specific DTH responses is correlated with a requirement to be able to respond to the carrier.

Carrier Requirements for the Elicitation of NP-specific DTH. The question that next arose was, could a similar Ir gene effect be demonstrated when the NP response was elicited by the various NP conjugates. To this end, various strains of mice were cyclophosphamide pretreated and primed with 100 μg of NP-BGG in CFA or with PBS in CFA. 7 d later, the mice were challenged with NP-BSA, NP-GLØ, or NP-GL.

As seen in Table II, NP-BSA was able to elicit a significant NP-specific DTH response in all strains of mice primed with NP-BGG. When NP-GLØ was used to elicit the response, the data clearly shows that this conjugate could also elicit an NP-specific response of comparable magnitude. This could be achieved even in strains of mice which could not be primed using the same batch of NP-GLØ, e.g., B10.GD and B10.S mice (Table I).

To further document this dichotomy in the capacity of a compound to induce and elicit DTH responses, we examined the ability of the nonimmunogenic compound, NP-GL, to elicit NP-specific DTH responses. As mentioned previously, mouse strains which respond to GL have not been identified (5, 6). The data in Table II demonstrate that the NP-specific response may be easily and efficiently elicited by NP-GL in NP-BGG primed mice. Furthermore, NP-BGG primed mice of either the B10.GD or B10.PL strains failed to respond when challenged with GLØ (data not shown). Thus, the response elicited by NP-GL is not a result of the recognition of GL determinants. Additional experiments were also carried out to show that the transfer of NP-specific

DTH required an H-2-linked gene homology even when NP-GL was used in the elicitation phase (data not shown). Thus, the elicitation of the NP-specific DTH response does not require use of an immunogenic carrier, but is nevertheless MHC restricted.

Discussion

These experiments were directed at a comparison between the carrier requirements for priming and elicitation of NP-specific, T-cell mediated DTH responses. Priming for NP-specific DTH responses was observed to require a genetic ability to respond to the carrier to which the NP hapten was conjugated. On the other hand, elicitation of an NP-specific response by appropriately primed mice could even be accomplished with NP-conjugates of nonimmunogenic carriers. Benacerraf and his colleagues (3) studied the antigenicity in guinea pigs of azobenzenearsonate (ABA) coupled to the immunogenic L or nonimmunogenic D stereoisomers of poly-(glu,ala,tyr) (GAT). Similar to the finding reported herein, they observed that ABA-D-GAT would not sensitize; however, ABA-D-GAT could nevertheless elicit weak ABA-DTH responses in ABA-L-GAT primed animals. Kipps et al.[1] also noted that GA determinants in GLA were unable to prime for a GA-specific DTH response in intermediate responder strain mice; nevertheless, GLA could elicit a DTH response in GA-primed mice.

Several possible mechanisms must be considered to explain the differences in the recipient for priming and elicitation of DTH reactivity. All interpretations must also account for the MHC restrictions which exist in this system. Thus, the various NP conjugates are presented on the surface of an antigen-presenting-cell in the context of an Ia molecule, as evidenced by the I-A homology requirement on transfer of DTH reactivity in such systems (1, 7). It is possible that the triggering threshold of the T cell for priming may have a higher thermodynamic energy requirement in the receptor interaction with the antigen-Ia complex, than the clonally expanded T cell demonstrates in the elicitation phase. Priming involves a complex series of differentiative and proliferative steps which could change the magnitude of the signal necessary to turn on the T cells at each step. A related explanation would postulate that the number of precursors recognizing a low affinity antigen-Ia complexes is so low that they would not expand to an extent sufficient to allow detectable priming. However, once the clones of NP-reactive T cells are expanded, a sufficient number of NP-specific cells could be triggered to give a detectable DTH response.

Another possible mechanism involves the introduction of the notion of maturation of affinity of the T-cell receptor in a manner analogous to that seen in the humoral antibody response. At the priming stage, one might envisage a T cell with a rather low affinity for a loose antigen-Ia complex. During the period of time between priming and elicitation, the T-cell receptor may mature to develop a higher affinity for the antigen-Ia complex. After receptor maturation, an NP-specific effector T cell might be able to cross-reactively recognize NP on a nonimmunogenic molecule.

A third more challenging possibility would implicate T cell-T cell interaction whereby priming of the effector NP-specific T-cell population is facilitated by a signal from a helper population of T cells recognizing a covalently linked carrier molecule. A helper effect on DTH was previously noted by Bullock et al. (8). Guinea pigs sensitized to ABA-tyrosine in CFA before immunization with ABA-protein developed

significantly stronger protein-specific DTH responses than those animals not previously immunized with ABA-T.

Finally, a less likely possibility is that the antigen-presenting cells involved in priming belong to a different functional subset from those involved in elicitation of DTH. The former would present the antigen immunogenically only if the carrier could be efficiently linked to an Ia molecule. The latter subset could present antigen to already matured T cells even in the absence of an immunogenic carrier moiety. At this point, there seems to be no compelling evidence for such a possibility.

In summary, we have presented several alternative mechanisms for explaining the observed dichotomy between the requirement for carrier recognition for priming, and lack of such a requirement for the elicitation of NP-specific DTH responses.

Summary

The genetic requirements of carrier recognition were examined in the priming and elicitation of hapten specific, T-cell mediated, delayed-type hypersensitivity (DTH) responses. It was shown that nitrophenyl acetyl-poly-(L-glu^{56}-L-lys^{35}-L-phe^{9}) (NP-GLØ) could prime for NP responses only in strains of mice which are Ir gene responders to GLØ. In contrast to this requirement, NP-GLØ could elicit an NP-specific response in NP-bovine gamma globulin primed mice, even in GLØ nonresponder strains. Furthermore, the nonimmunogenic molecule, NP-GL, could elicit an NP-specific DTH response in animals primed with NP on an immunogenic carrier.

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