HAPTEN-SPECIFIC T-CELL RESPONSES TO 4-HYDROXY-3-NITROPHENYL ACETYL

I. Genetic Control of Delayed-Type Hypersensitivity by V_H and I-A-Region Genes*

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The biochemical nature of the T-cell antigen recognition structure and its relationship to serum antibody is still an important unresolved issue in immunology. Several independent lines of evidence indicate that immunoglobulin V-region products are involved in the cell-surface T-cell receptor (1–5). Most of the evidence supporting this concept has been obtained by using anti-idiotypic antisera as probes to analyze the biological and biochemical properties of such receptors. The present experiments describe the influence of genes in the V_H-region on the specificity of selected T-cell responses. The approach taken here was based on a genetic analysis of the fine specificity of the T-cell response to the (4-hydroxy-3-nitrophenyl)acetyl (NP)[1] hapten. Recent work from several laboratories on the primary anti-NP antibody response has revealed the presence of genetic V-region markers in these anti-hapten antibodies by both anti-idiotypic and fine-specificity characterization (6–8). It was found that the primary anti-NP antibody response in most mice of the $Ig1^b$ allotype has a peculiar heteroclitic fine specificity (6), i.e., these antibodies bind an analogue of the immunizing hapten, (4-hydroxy-5-iodo-3-nitrophenyl)acetyl (NIP), with greater affinity than the immunizing hapten, NP, itself. The primary anti-NP antibodies from such heteroclitic strains share common, strain-specific clonotypes as shown by isoelectric focusing (9). Furthermore, two laboratories have prepared anti-idiotypic antibodies, one, apparently combining site specific, as evidenced by the ability of hapten to inhibit anti-idiotype binding of the idiotypic antibody, and the other, hapten noninhibitable (7, 8). Both of these anti-idiotypic reagents have been used to identify at least one germ-line gene which maps to the V_H-region of $Ig1^b$ heavy-chain linkage group. This gene has been termed V_HNP^b (7, 10). The idiotype-positive antibody comprises the predominant antibody population of the primary anti-NP response of C57BL/6 mice (>85%) and also of F_1 hybrid mice between C57BL/6 and an idiotype-

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[1] *Abbreviations used in this paper;* BGG, bovine gamma globulin; BSA, bovine serum albumin; CFA, complete Freund's adjuvant; DNP, dinitrophenyl hapten; DTH, delayed-type hypersensitivity; MGG, mouse gamma globulin; MHC, major histocompatibility complex; NIP, (4-hydroxy-5-iodo-3-nitrophenyl)acetyl hapten; NP, (4-hydroxy-3-nitrophenyl)acetyl hapten; PABA, *p*-azobenzoate; PBS, phosphate-buffered saline.

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negative strain (8). Finally, the idiotype-positive antibody expresses the λ-light chain as its predominant light-chain species, and all detectable heterogeneity in the idiotype-positive population could be accounted for by the heterogeneity among the heavy chains (7, 8). SJL, despite producing $Ig1^b$ allotype-positive antibody, has been shown to produce a nonheteroclitic, idiotype negative, primary anti-NP response. The V_HNP^b gene, however, is thought to be present in SJL, as is shown by the demonstration of idiotype production in primary anti-NP response of F_1 crosses of SJL with nonheteroclitic strains, (CBA × SJL)F_1 mice (7), or (BALb/c × SJL)F_1 mice (8). Thus, the primary anti-NP antibody response is associated with a characteristic fine-specificity marker, clonotype, λ-light chain bearing immunoglobulin and idiotypic markers.

These characteristics of the V-region markers of anti-NP antibodies have also been used in characterization of NP-binding receptor material isolated from enriched populations of NP-primed splenic B- and T-lymphocytes. Antigen-binding material was isolated by adsorption of NP-specific T lymphocytes to hapten-conjugated nylon wool, followed by a temperature shift to release the cells, and elution of the receptor material with acidic buffer or free hapten. Amounts of receptor material isolated from C57BL/6 splenic lymphocytes lacking immunoglobulin C-region determinants of either heavy or light chains, correlated with the relative number of T cells. It was concluded that this material was of T-cell origin (11). Genetic analysis of the anti-NP responses of (C57BL × CBA/J)F_2 mice, indicated that the putative T-cell receptor material was heteroclitic if, and only if, the material originated from animals expressing the $Ig1^b$ allotype. Furthermore, an identical correlation was found between the heavy-chain allotype and the presence of the idiotype on this material. It was thus concluded that in this system the T-cell receptor for antigen bears identical V_H-region markers as those found on immunoglobulin of the same specificity (11, 12).

In addition to V_H-region markers, another characteristic property of T lymphocytes is their functional specificity for antigens of the major histocompatibility complex. The dual specificity of T cells indicates the contribution of the products of two distinct chromosomes to the functional specificity of T cells and inspired the hypothesis by several investigators of two receptors on T lymphocytes, one for the nominal antigen and the other for the major histocompatibility complex MHC-gene product. We felt that the study of a functional T-cell response, such as hapten-specific delayed-type hypersensitivity in the NP-NIP system should provide information in the appropriate mouse strains on the expression of V_H genes on T cells together with receptors specific for MHC-gene products.

The experiments reported here establish the V_H-region control of the fine specificity of delayed-type hypersensitivity (DTH) against NP, the T-cell nature of this phenomenon, and an independent MHC-linked restriction on the function of these T cells. The evidence presented below indicates that at least two distinct genetic loci are involved in determining the specificity of functional DTH T cells.

Materials and Methods

Mice. C57BL/6, BALB/c, (BALB/c × C57BL/6)F_1, (C57BL/6 × C3H)F_1, B10.A, A/J, B10.BR, SJL, and C3HeB/FeJ mice were purchased from The Jackson Laboratory, Bar Harbor, Maine. C.B-20, BAB/14, B10.A(4R), B10.A(5R), and CBA(M523) mice were bred in the animal facilities of Harvard Medical School. Mice were used at 2–20 mo of age, and were maintained on laboratory chow and acidified, chlorinated water ad libitum.

Antigens. Two times recrystallized NP-OSuccinamide and NIP-OSuccinamide were purchased from Biosearch (San Rafael, Calif.). NP and NIP conjugates of bovine serum albumin (BSA) and bovine gamma globulin (BGG) were prepared as follows. BSA and BGG (Sigma Chemical Co., St. Louis, Mo.) were dissolved in a pH 9.0, 0.2 M $NaHCO_3$-NaOH buffer, to achieve a final protein concentration of 20 mg/ml. The active succinamide dissolved in dioxane at 25 mg/ml for NP conjugates, and 33 mg/ml for NIP conjugates was added at room temperature. For each milliliter of carrier solution to be conjugated, 80 μl of the succinamide solution was added dropwise. The reaction was allowed to proceed for 18 h at 4°C, and the resulting compounds were dialyzed extensively against borate buffered saline, pH 8.4. The final conjugation ratios were NP_{11}-BSA, NP_{17}-BGG, NIP_9-BSA, and NIP_{18}-BGG.

Immunization and Challenge. All animals described below, except adoptive transfer recipients, were pretreated with 20 mg/kg cyclophosphamide in 0.2 ml phosphate-buffered saline (PBS), injected i.p., 3 days before immunization. The NP-BGG or NIP-BGG antigens were emulsified in complete Freund's adjuvant (CFA) (Difco Laboratories, Detroit, Mich.) containing *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. Donors for adoptive transfer experiments received an additional two 0.1-ml s.c. injections near the forelimbs.

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

Adoptive Transfer Experiments. Draining lymph nodes from donors primed 7 d earlier, were removed and a single-cell suspension was prepared by gentle teasing in cold Eagle's minimum essential medium. These cells were washed twice, counted, and transferred intravenously to naive recipients.

In several experiments, the cells to be transferred were twice treated with AKR anti-C3H antiserum (anti-Thy 1.2) and rabbit complement as previously described (13).

Histology of Footpad Responses. The feet of animals in which responses were elicited 7 d after immunization were removed and placed in buffered 10% formalin. Hematoxylin and eosin-stained sections of the decalcified feet were prepared by Ms. M. Hagney of Harvard Medical School.

Statistical Analysis. Analysis of average differences of swelling obtained was performed with the Wang Programmable computer. 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 indicated otherwise.

Results

Induction of NP-Specific DTH Responses. When animals were pretreated with 20 mg/kg cyclophosphamide, followed 3 d later by subcutaneous priming with 100 μg NP-BGG in CFA, NP-specific DTH could be elicited in response to challenge with NP conjugated to a heterologous carrier, BSA. The DTH response was characterized by a delayed onset of footpad swelling which peaked at 20–36 h and by a mononuclear cell infiltrate (data not shown). As shown in Table I, A/J, CBA(M523), BALB/c, and C3H mice which were classified as nonheteroclitic strains at the antibody level made NP-specific DTH responses after priming with NP-BGG and challenge with NP-BSA. The specificity of these responses is evidenced by the inability of NP-BGG primed mice of the A/J, CBA(M523), BALB/c, and C3H strains to display significant levels of DTH after challenge with NIP-BSA. The latter findings imply that BSA is not significantly cross-reactive with BGG, and that the DTH response is truly NP specific.

TABLE I

*The Cross-Reactivity of NP with NIP in DTH Responses of Different Strains of Mice**

Strain	Heavy-chain haplotype	Immunogen	DTH challenge	
			NP-BSA‡	NIP-BSA
A/J	e	NP-BGG	45.0 ± 2.9	8.8 ± 2.2
		NIP-BGG	NT	54.8 ± 4.9
		CFA	13.8 ± 1.8	10.2 ± 3.8
CBA(M523)	j	NP-BGG	32.6 ± 2.2	9.5 ± 4.8
		NIP-BGG	NT	37.5 ± 2.1
		CFA	6.6 ± 2.7	5.6 ± 2.8
BALB/c	a	NP-BGG	68.5 ± 7.2	5.5 ± 3.3
		NIP-BGG	NT	60.5 ± 2.5
		CFA	8.0 ± 5.4	5.5 ± 3.7
C3H	j	NP-BGG	87.0 ± 5.8	20.3 ± 3.6
		NIP-BGG	NT	83.8 ± 5.4
		CFA	10.2 ± 1.4	16.0 ± 2.7
C57BL/6	b	NP-BGG	82.0 ± 10.0	57.0 ± 5.8
		CFA	16.3 ± 5.5	5.0 ± 3.9
B10.A	b	NP-BGG	67.0 ± 5.8	63.5 ± 1.6
		NIP-BGG	NT	56.0 ± 2.6
		CFA	12.3 ± 4.3	4.8 ± 2.6
(C57BL/6 × C3H)F_1	b/j	NP-BGG	64.4 ± 3.7	51.5 ± 3.8
		NIP-BGG	NT	77.3 ± 10.4
		CFA	7.4 ± 0.8	6.7 ± 1.7
(BALB/c × C57BL/6)F_1	a/b	NP-BGG	39.4 ± 3.8	35.2 ± 4.1
		NIP-BGG	NT	42.4 ± 2.5
		CFA	4.0 ± 2.7	1.2 ± 3.0

NT, not tested.

* Animals were treated with 20 mg/kg cyclophosphamide. 3 d later they were primed subcutaneously in two sites with a total of 100 μg NP-BGG or NIP-BGG in CFA. 7 d after priming, the animals were challenged with the appropriate challenge antigen. Swelling of challenge foot compared with control foot was measured 24 h after challenge.

‡ Responses of a minimum of four animals per group in 10^{-4} inches. Expressed as mean ± 1 SE.

In addition, NIP-specific DTH responses can be induced in each of these strains of mice if they are primed with NIP-BGG (Table I).

Fine Specificity of the DTH Response Induced with NP. In view of the heteroclitic antibody responses to NP in appropriate strains of mice, the ability of various strains carrying the $Ig1^b$ allotype to demonstrate cross-reactive DTH responses to NIP, after NP priming, was tested. As indicated in Table I, C57BL/6 and B10.A mice primed with 100 μg of NP-BGG produce DTH responses after challenge with NP-BSA. However, the DTH reactions in these strains are highly cross-reactive with NIP-BSA. The magnitude of the cross-reactive NIP responses were comparable to the NP responses. As indicated above, A/J, C3H, BALB/c, and CBA(M523) strain mice do not produce a significant cross-reactive NIP response when they have been primed with NP. This is not a result of an inability to respond to NIP; thus, A/J, CBA(M523), BALB/c, and C3H mice make significant NIP DTH responses to NIP-BSA when primed with NIP-BGG. The difference in the specificity of the DTH response to NP are not controlled by genes in the MHC region, because B10.A and A/J mice share the $H\text{-}2^a$ haplotype, yet differ in their ability to cross react with NIP. The data

TABLE II

*Cell Transfer of NP-Specific DTH in C57BL/6 Mice**

Donor cells	Treatment	DTH challenge	
		NP-BSA	NIP-BSA
NP immune	—	48.0 ± 7.9‡	49.7 ± 9.3
control	—	5.5 ± 3.4	2.3 ± 2.3
NP immune	NMS + C	31.0 ± 8.0	NT
NP immune	Anti-Thy 1.2 + C	7.3 ± 2.6	NT

NT, not tested.

* Immune C57BL/6 donor animals were pretreated with cyclophosphamide 3 d before priming with NP-BGG in CFA. 7 d after priming 2×10^7 draining lymph node cells were transferred i.v. to naive syngeneic recipients. Control mice received a mixture of 2×10^7 lymph node and spleen cells obtained from C57BL/6 mice which had received cyclophosphamide 3 d before immunization with CFA.

‡ Arithmetic mean ± 1 SE of groups consisting of four or more animals. Expressed in units of 10^{-4} inches.

presented in Table I also demonstrate that F_1 hybrids between a strain which cross-reacts to NIP and strains which do not exhibit the NIP cross-reactivity, namely (C57BL/6 × C3H)F_1 and (BALB/c × C57BL/6)F_1 hybrids, exhibited significant cross-reactive NIP responses after NP priming. Thus, the NIP cross-reactivity in DTH responses is the dominant phenotype in the F_1 combinations tested.

Cellular Requirements for Transfer of DTH. The next series of experiments were performed to demonstrate that the responsiveness to NP compounds was transferable and T-cell dependent (Table II). In the first experiment, 2×10^7 draining lymph node cells from cyclophosphamide pretreated, NP-BGG-primed animals were transferred intravenously to naive syngeneic recipients. Control animals received a transfer of lymphoid cells from cyclophosphamide pretreated, CFA-primed animals. Only those animals which received lymphoid cells from NP-primed animals showed significant reactivity to challenge with NP-BSA. Furthermore, the cross-reactive DTH response to NIP, noted in B6 mice, was transferable with NP-immune cells (Table II). Thus, lymph node cells are sufficient to transfer this DTH reactivity.

The second experiment was carried out to investigate the T-cell dependence of the ability to transfer the delayed hypersensitivity reaction. Accordingly, 2×10^8 NP immune lymph node cells were treated with either normal mouse serum plus complement or anti-Thy 1.2 antiserum plus complement, before transfer to naive syngeneic hosts. The number of cells recovered after treatment were only sufficient to transfer and check reactivity with NP-BSA. As seen in Table II, anti-Thy 1.2 plus complement treatment significantly ($P < 0.05$) diminished NP responses to levels comparable to the background reactivities seen in naive animals. Thus removal of T cells abrogates ability of immune lymph node cells to tranfer DTH reactivity.

Genetic Mapping of NP Cross-Reactivity on DTH. To map the genes responsible for the strain differences seen in the ability of NP-immune mice to respond to the cross-reactive NIP hapten, mice congeneic for the immunoglobulin heavy-chain linkage group were used (Table III). BALB/c mice have heavy-chain constant region and heavy-chain variable region markers of the $Ig1^a$ allotype. C.B-20 is congeneic at these loci with BALB/c, and expresses the $Ig1^b$ constant heavy chain and variable heavy-chain markers derived from the C57BL/Ka strain (14). The data indicates the BALB/c NP primed mice do not make cross-reactive NIP responses, whereas C.B-20 mice do. Thus, genes in the heavy-chain linkage group control ability to cross-react to NIP.

TABLE III

*Genetics of NP Cross-Reactivity with NIP in DTH Responses**

Strain	Heavy-chain haplotype		Immunogen	DTH challenge	
	C_H	V_H		NP-BSA‡	NIP-BSA
BALB/c	a	a	NP-BGG	68.5 ± 7.2	5.5 ± 3.3
			NIP-BGG	NT	60.5 ± 2.5
			CFA	8.0 ± 5.4	5.5 ± 3.7
C.B-20	b	b	NP-BGG	45.6 ± 3.0	52.5 ± 4.8
			CFA	−4.0 ± 4.0	8.0 ± 2.0
BAB/14	b	a	NP-BGG	48.8 ± 6.0	2.5 ± 2.6
			NIP-BGG	NT	41.5 ± 6.4
			CFA	6.0 ± 3.3	3.0 ± 2.5
SJL	b	b	NP-BGG	59.0 ± 2.0	54.8 ± 1.4
			NIP-BGG	NT	54.5 ± 5.8
			CFA	8.0 ± 2.0	5.0 ± 1.7

NT, not tested.

* Animals were treated with 20 mg/kg cyclophosphamide. 3 d later they were primed subcutaneously in two sites with a total of 100 μg NP-BGG or NIP-BGG in CFA. 7 d after priming, the animals were challenged with the appropriate challenge antigen. Swelling of challenge foot compared with control foot was measured 24 h after challenge.

‡ Responses of a minimum of four animals per group in 10^{-4} inches. Expressed as mean ± 1 SE.

In an effort to determine whether genes in the V_H- or C_H-genetic regions control this trait, another congeneic strain, BAB/14, was examined. The BAB/14 mice carry a recombinant heavy-chain linkage group. The C_H genes are derived from the $Ig1^b$ linkage group, and most of the V_H genes are derived from the BALB/c parent, including genes coding for the NP idiotype in this strain (15, 7). It was found that mice from this strain do not make a significant cross-reactive response to NIP, with only NP priming. This indicates that V_H-region genes exert control over the expression of the ability to make cross-reactive NIP DTH responses.

Finally, mice of the SJL strain were studied. As indicated in the introduction, these mice do not make heteroclitic antibody despite having the proper C_H-linkage group. In the DTH assay, however, it was found that SJL mice do make significant cross-reactive NIP responses when primed with NP-BGG.

Genetic Restriction on Transfer of NP-Specific DTH. To investigate the role of the MHC in the ability to transfer delayed hypersensitivity to NP, a series of allogeneic transfer experiments were performed. Donor mice were cyclophosphamide pretreated and primed subcutaneously in four sites with 200 μg NP-BGG in CFA. 7 d later, 3×10^7 draining lymph node cells were transferred intravenously into recipients of various mouse strains. 1 h after transfer, the recipient mice were challenged in the footpad with either NP-BSA or NIP-BSA. Swelling was measured 24 h after challenge (Table IV).

Lymphoid cells from C57BL/6 mice sensitized to NP transferred reactivity to both NP and NIP, when transferred into H-2-identical hosts, C57BL/10, or into semi-allogeneic hosts, (C57BL/6 × C3H)F_1. On the other hand, transfer to a completely H-2 nonidentical host, B10.BR, was unsuccessful. The reciprocal experiment, namely B10.BR transfer, was successful into syngeneic and (C57BL/6 × C3H)F_1 mice. The immune B10.BR lymphoid cells were incapable of transferring DTH reactivity to a

TABLE IV

*Ability of NP-Primed Cells to Transfer DTH Reactivity to Different Strains of Mice**

Recipient	H-2-region formulae (K,IA,IB,IJ,IE,IC,S,D)	Donors of immune cells transferred			
		C57BL/6‡		B10.BR‡	
		NP-BSA§	NIP-BSA	NP-BSA	NIP-BSA
C57BL/10	b,b,b,b,b,b,b,b	51.6 ± 6.3 ‖	47.7 ± 10.4 ‖	7.9 ± 2.4	5.2 ± 4.3
(C57BL/6 × C3H)F_1	b,b,b,b,b,b,b,b / k,k,k,k,k,k,k,k	57.6 ± 6.5 ‖	62.5 ± 4.9 ‖	43.3 ± 6.5 ‖	53.7 ± 7.0 ‖
B10.BR	k,k,k,k,k,k,k,k	10.6 ± 5.0	5.5 ± 4.4	31.9 ± 4.6 ‖	22.0 ± 5.5 ‖
B10.A(4R)	k,k,b,b,b,b,b,b	15.0 ± 5.2	5.5 ± 3.7	48.0 ± 5.6 ‖	46.9 ± 9.0 ‖
B10.4(5R)	b,b,b,k,k,d,d,d	53.1 ± 6.9 ‖	60.0 ± 4.1 ‖	NT	NT
CBA(M523)	ka,k,k,k,k,k,k,k	NT	NT	52.5 ± 7.0 ‖	47.6 ± 7.2 ‖

NT, not tested.

* Donor animals were treated with cyclophosphamide and 3 d later injected subcutaneously with a total of 200 μg NP-BGG in a 0.4-ml 50% CFA solution, in four separate sites. 7 d after priming, 3×10^7 draining lymph node cells were transferred to naive recipient animals. The recipients were challenged 1 h after transfer.

§ Minimum of four recipients per group. Arithmetic mean ± SE. Units of 10^{-4} inches.

‡ Backgrounds for each of the above strains using transfer of cyclophosphamide pretreated, CFA-primed donors of lymph node and spleen cells were: C57BL/6-NP-background response 8.4 ± 4.8, NIP-background response 13.8 ± 5.1; B10.BR-NP-background response 4.8 ± 1.5, NIP-background response 8.2 ± 3.3.

‖ $P < 0.01$.

congeneic H-2 nonidentical host, C57BL/10. In addition, (B6 × A)F_1 NP-immune cells could transfer DTH reactivity to C57BL/6 parental strain mice; thus the cells were not simply rejected (data not shown). Thus, ability to transfer NP-specific DTH is MHC linked.

To map the genetic subregion of the MHC which controls successful transfer of DTH, several mouse strains with recombinant or mutant MHC regions were examined with respect to their ability to successfully mount a response using the transferred cell populations. When C57BL/6 (H-2^b) NP-immune lymph node cells were transferred into B10.A(4R) (kkbbbbbb) mice which share the I-B, I-J, I-E, I-C, S, and D regions with the donor C57BL/6 strain, no transfer of NP reactivity was noted. However, when the same cells were transferred into B10.A(5R) mice (bbbkkddd), which share the K, I-A, and I-B regions with the B10 donor strain, successful transfer of NP reactivity was achieved. Similar results demonstrating that genes in the K and/or I-A were required for successful transfer were obtained using different strain combinations. Thus, immune B10.BR (H-2^k) lymphoid cells transferred NP-specific reactivity to B10.A(4R). The ability of immune B10.BR lymphoid cells to transfer immunity to the CBA(M523) mice, which possess a mutant H-2K allele, indicates that identity at H-2K is not required for transfer of NP DTH reactivity. Furthermore, the ability to transfer immunity to the CBA(M523) strain which also differs from the B10.BR donor strain at numerous non-H-2 loci, including the C_H and V_H loci, indicates homology for these loci is not required for transfer of NP DTH reactivity. In all cases in which NP reactivity was successfully transferred, concomitant cross-reactivity to challenge with NIP in the recipient was noted.

Discussion

Specific DTH responses to the NP and NIP haptens could be induced in various strains of mice after cyclophosphamide pretreatment and immunization with NP or

NIP conjugated to bovine gamma globulin. Evidence for the existence of hapten-specific T cells has been found in other systems. Yamashita and Kitagawa (16) found that dinitrophenyl-mouse gamma globulin (DNP-MGG)-immunized mice developed T-helper cells which provided help in response to DNP-keyhole limpet hemocyanin and that *p*-azobenzoate (PABA)-BGG-immunized mice developed radioresistant T cells capable of cooperating with DNP-primed B cells in response to DNP-MGG-PABA (16). Hamaoka et al. (17) demonstrated that PABA-specific helper T cells could be tolerized by PABA-copolymer of D glutamic acid and D-lysine. Cosenza et al. (3) were able to prime PC-specific helper T cells by pretreating the donor with anti-idiotypic antibodies. Thus hapten-specific helper T-cell function has been demonstrated in several systems.

The use of cyclophosphamide in the induction of the hapten-specific DTH responses described above was motivated by the following considerations. The differential sensitivity of B cells and suppressor T cells to cyclophosphamide treatment have been documented (18–21). In addition, Askenase et al. (20) have shown an augmentation of DTH responses by low doses of cyclophosphamide. The NP-specific DTH response was a classical DTH reaction, characterized by edema, induration, and a mononuclear cell infiltrate. Futhermore, the time course of the response was consistent with a classical DTH reaction; swelling was not detectable until 12–16 h after challenge, and reached its maximal magnitude at 20–36 h. Finally, the immunity could be adoptively transferred by lymphoid cells, was T-cell dependent, and MHC restricted.

The NP system was chosen for analysis, because the genetics of the fine specificity of the antibody response have been well documented (6–10). Furthermore, Krawinkel et al. (11) demonstrated that a putative receptor material demonstrating the same fine specificity as anti-NP antibody could be isolated from enriched populations of T cells. Because the question of which functional subpopulations of T cells bore this receptor remained unresolved, the present experiments were initiated. The present data demonstrate that a gene(s) coded for by the V_H-region controls the fine specificity of the DTH response, and suggest that at least one T-cell population involved in the DTH response bears a V_H-coded receptor. In strains lacking the $Ig1^b$ allotype, priming with NP-BGG induced a hapten-specific DTH response to a challenge of NP coupled to a different carrier, BSA. That this response was NP specific, and not a result of a cross-reactivity between BGG and BSA proteins used for priming and challenge, was demonstrated by the inability of NIP-BSA to elicit a DTH response in similarly primed animals. Furthermore, we demonstrated that animals primed with NIP-BGG could give a NIP DTH response when challenged with NIP-BSA. In those strains possessing the $Ig1^b$ heavy-chain linkage group, such as the C57BL/6 and B10.A strains, the fine specificity of the NP-primed DTH response was clearly different. Thus, NP-primed C57BL/6 or B10.A mice primed with NP-BGG and challenged with NIP-BSA, developed DTH reactions with almost the same magnitude as similarly primed animals responding to NP-BSA. In addition, the fine specificity of DTH-reactivity pattern of (NIP cross-reactive × noncross-reactive)F_1 hybrids (C57BL/6 × C3H)F_1 and (BALB/c × C57BL/6)F_1 were like those of the NIP cross-reactive C57BL/6 parental mice. Thus, ability to respond cross-reactively to NIP was inherited in a dominant or codominant fashion. It should be noted that the conditions used to observe hapten-specific DTH responses had been optimized in the present study to demonstrate maximal DTH reactivity. Under more limiting conditions, such as observation at 36–48 h after DTH challenge, the cross-reactive NIP response appeared

larger and more consistent than the response to the homologous NP hapten (data not shown). Thus, we should term the NP-induced NIP cross-reactive DTH response, heteroclitic.

A detailed genetic study of the control of the NIP cross-reactive or heteroclitic fine-specificity characteristics was undertaken. From the data presented in Tables I and III, in which MHC-identical mouse strains, such as B10.A and A/J or BALB/c and C.B-20 demonstrated different DTH fine-specificity patterns, it was concluded that genes in the MHC did not control the expression of DTH-heteroclitic NP responses. The BALB/c and C.B-20 are congeneic with respect to the heavy-chain linkage group. BALB/c mice express the $Ig1^a$ allotype, whereas C.B-20, which are theoretically identical with respect to other background genes, express $Ig1^b$ genes in the heavy-chain linkage group. It was found that C.B-20 mice make heteroclitic DTH responses to NP, whereas BALB/c mice were unable to do so. These data with congeneic mice confirm the strain-distribution data and indicate that a gene linked to the heavy-chain linkage group controls expression of the heterocliticity of DTH responses to NP. To more precisely localize the gene(s) responsible for the ability to make such heteroclitic responses, the BALB/c congeneic, BAB/14 strain, which carries a recombinant haplotype in which crossing over occurred between the C_H genes derived from $Ig1^b$ and the V_H genes derived from the $Ig1^a$ allele of the BALB/c parent, were tested. At the humoral level, BAB/14 mice do not express the V_HNP^b gene, and, thus, do not produce heteroclitic antibody responses (7, 8). In the DTH assay described above, BAB/14 mice, when primed with NP, also failed to cross-reactively respond to NIP, and thus were not heteroclitic. The data, therefore, indicate a gene in the V_H-region controls the expression of the heteroclitic DTH, T-cell dependent, response.

The presence of V_H-region products has been described in other systems (1–5). V_H-region products have been identified indirectly on T-helper cells (22, 23), alloreactive T cells (24), and T-cell derived suppressor (25, 26) and helper (27, 28) factors. In these studies, similar V_H-region products were also found on immunoglobulins of the same specificity. This study identifies V_H-gene expression on a functional class of T cells without the aid of serological reagents. Moreover, genetic mapping of the V_H-region markers of the heteroclitic T-cell DTH response is to the same region as the immunoglobulin produced in the primary heteroclitic anti-NP response. The simplest explanation for this is that the T and B cell express some V_H-region products coded for by the same gene(s), in their respective anti-NP response. Thus, the evidence presented indicates that at least one T cell in the DTH pathway for anti-NP responses has a V_H-coded receptor, sharing fine-specificity characteristics with the B-cell receptor for the same specificity.

The expression of the heteroclitic fine specificity patterns is not absolutely identical at the T- and B-cell levels. The one exception note was the response of SJL mice which was particularly informative. SJL mice fail to produce heteroclitic antibody in response to NP, despite the fact that this strain bears the $Ig1^b$-linkage group (7, 8). SJL mice also produce very low levels of λ-light chains (29). It has been postulated that production of adequate levels of λ-chains is necessary for expression of the gene-controlling heteroclitic anti-NP antibody (7, 8). The fact that SJL mice do have the gene which controls heteroclicity, V_HNP^b, is indicated by studies showing that (BALB/c × SJL)F_1 (7) and (CBA × SJL)F_1 (8) hybrid mice (hybrids between SJL mice and mice of strains with non-$Ig1^b$ heavy-chain linkage groups) make heteroclitic

primary anti-NP antibody responses. This is presumably a consequence of the functional complementation between the V_HNP^b gene of the SJL and the functioning λ-chain production mechanism contributed by the other parent. When the SJL strain was tested in the DTH assay, it was found that SJL mice made cross-reactive NP-induced DTH responses (Table III). Thus, SJL strain mice express the V-gene marker for T-cell heteroclicity. Several conclusions are implied. First, because SJL mice do not express heteroclitic anti-NP antibody, but do make heteroclitic T-cell anti-NP DTH responses, heteroclitic DTH T cells probably produce their own functional antigen-specific receptor. Second, the results of the SJL strain implies that either T cells can endogenously produce λ-light chains for use in making a heteroclitic T-cell receptor, or T cells do not make use of a λ-light chain in construction of a heteroclitic T-cell receptor.

MHC restriction on T-cell function has been demonstrated for cytotoxic T cells (30–33), DTH-reactive cells (34, 35), and for T-cell-B-cell collaboration (36). In an effort to demonstrate similar MHC restriction on the population of NP-specific DTH-reactive T cells, a series of acute adoptive transfer experiments were performed. The first point of note was that whenever NP reactivity was successfully transferred from an NP-primed DTH-heteroclitic strain, a simultaneous transfer of NIP cross-reactivity occurred. Second, K- and I-A-region homology between donor and recipient was sufficient to allow transfer of DTH reactivity. This was shown by the ability of C57BL/6 cells to transfer DTH reactivity to B10.A(5R), but not to B10.A(4R). B10.A(4R) is homologous with C57BL/6 across the H-2 complex, except at K and I-A, whereas B10.A(5R) is H-2 homologous with C57BL/6 at the K, I-A, and I-B regions. In addition, it appears that the I-A region may be critically involved because disparity at H-2K was not sufficient to prevent transfer of NP-specific DTH reactivity from B10.BR to CBA(M523) (although we must be cautious when using an H-2K mutant to define disparity). It is also remarkable that this transfer was successful despite the disparate background genes, including C_H and V_H, of the recipient as compared with the donor. These results imply that homology at (*a*) K and I-A or D and I-A, or (*b*) I-A alone are sufficient to allow DTH transfer of NP reactivity, and that homology at other loci, including C_H- and V_H-region genes, are not required. These findings are in accord with the conclusions of Miller et al. (34) who previously demonstrated that, for protein antigens, DTH reactivity could be transferrred between I-A-subregion homologous strains of mice.

Moreover, these experiments demonstrate that the functional specificity of the primed NP-specific DTH T-cell population is controlled by genes mapping in the V_H and H-2 complexes. There are at least two likely possibilities explaining the apparent dual recognition by the T cell of NP-haptenic determinants and H-2-coded determinants. One explanation would be that different T-cell populations separately express the antigen reactivity controlled by gene products of the V_HNP^b gene, and MHC restriction. These two cell types would have to communicate with each other to evoke a DTH response. This, however, is an unlikely possibility on the basis of the accumulated evidence in other T-cell responses (37). The second major and more likely possibility to explain the described phenomenon is that there are T cells in the primed T-cell population which express, simultaneously, V_HNP^b gene products and, independently, express MHC restriction. The data are most consistent with two binding sites for DTH-specific T cells in this system. Whether such cells have one or

two structures on their surface for identifying the two specificities, cannot be definitely concluded from currently available data. These studies were aimed at defining the genetic control of the heteroclitic DTH T-cell response, and its MHC restriction. Whether similar heteroclitic specificity can be seen in other T-cell functional responses to NP, such as T-dependent help, suppression or cytolysis, remains to be investigated.

Summary

Hapten-specific delayed-type hypersensitivity (DTH) was induced in several strains of mice. (4-hydroxy-3-nitrophenyl)acetyl-bovine gamma globulin (NP-BGG)-primed mice which did not bear the $Ig1^b$ heavy-chain linkage group made a NP-specific DTH response when challenged with NP bovine serum albumin (BSA) and failed to respond to challenge with (4-hydroxy-5-iodo-3-nitrophenyl)acetyl-bovine serum albumin (NIP-BSA). Strains of NP-BGG-primed mice bearing the $Ig1^b$ allotype, including SJL, responded to challenges of either NP-BSA or NIP-BSA. F_1 hybrids between a cross-reactive strain, C57BL/6, and two other noncross-reactive strains were cross-reactive.

Genetic mapping of the NIP-cross-reactive DTH response localized the trait to the V_H-region of the $Ig1^b$ heavy-chain linkage group. The fine-specificity pattern of the T-cell anti-NP response, and the genetic mapping of this trait, were analogous to the reported fine specificity and mapping data of the humoral heteroclitic anti-NP response.

Adoptive transfer studies on the ability to transfer NP-specific DTH between various strain combinations showed that the T-cell donors and the recipient must have homology for at least the I-A subregion. Whenever NP-specific reactivity was transferred from a strain which cross-reactively responded to NIP, the recipient also responded to both NP and NIP. The implications of the control of NP-primed DTH-reactive populations of T cells by two distinct genetic regions, V_H and H-2, were discussed.

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