HELPER T CELL RECOGNITION OF THE VARIABLE DOMAINS OF A MOUSE MYELOMA PROTEIN (315)

Effect of the Major Histocompatibility Complex and Domain Conformation *

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A basic assumption of Jerne's network theory (1) is that the immune system of an individual can recognize its own antigen receptors. Because this concept opens many possibilities for immunological manipulation (2), it is important to ascertain whether the immune system recognizes its own immunoglobulin (Ig) variable (V) 1 domains in a way that conforms to the experience gained with conventional extrinsic protein antigens.

To this end we have been studying the antigenicity of the hapten- (dinitrophenyl [DNP]-lysine) binding IgA mouse myeloma protein 315. The amino acid sequences of the entire L-315 and the V region of the H-315 (VH-315) chains have been determined (3, 4). From these data it is clear that L-315 belongs to the rare λ2-type, found in ~1% of normal Ig in most strains of mice (5). The mouse λ genes are arranged in two clusters on the chromosome: VL1, Jλ, Cλ, J1, C1 and VH2, Jκ, Cκ, Jκ, Cκ (6). Of the three Ig multigene families in the mouse, the λ gene family thus has the smallest number of V genes, i.e., only two.

The immune response of BALB/c mice to M315 was first studied by Sirisinha and Eisen (7), who demonstrated that M315 mixed with Freund's complete adjuvant elicited antibodies specific for an antigenic determinant that was only expressed when H-315 and L-315 chains were assembled, and that was intimately associated with the hapten binding site. Work carried out in this laboratory (8) has revealed that immunization with affinity-labeled M315 (DNP-lysine covalently attached in the combining site) induced antibodies that recognized determinants which also required assembled H + L chains, but which were located outside the combining site, because the antibodies formed complexes with M315 in the presence of a high concentration of DNP-lysine. Furthermore, the free form of native λ2-315 evoked antibodies that bound free but not assembled λ2 of the complete M315 (9). In contrast, carrier-specific helper T cells primed with free λ2-315 or its V domain (Vλ2) responded to a boost with the complete M315 (10), suggesting that cellular and humoral immunity elicited

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Supported by the Norwegian Research Council for Science and the Humanities, the Norwegian Cancer Society and the Norwegian Society for Fighting Cancer.

Abbreviations used in this paper: BSA, bovine serum albumin; DNP, dinitrophenyl; Fv-315, fragment containing the two variable domains of M315; H-2, the MHC of the mouse; MHC, major histocompatibility complex; M315, myeloma protein produced by MOPC-315; NIP, 4-hydroxy-3-iodo-5-nitrophenylacetyl; Ir, immune response; VH, the variable domain of the M315 heavy chain; Vλ, the variable domain of the M315 light chain.
by the isolated $\lambda_2$-315 recognize different antigenic sites. $V_{H}$-315 was distinct from $V\lambda_2$ in the sense that it was not immunogenic for helper T cells in BALB/c mice (10).

In a previous report (11) we presented evidence that the responses of conventional helper T cells primed with the free forms of $V_{H}$-315 and $V\lambda_2$-315 are controlled by genes linked to the H-2 complex. In the present work these observations are confirmed and extended to include mapping of the immune response (Ir) gene for $V_{H}$-315. Mapping of the Ir gene for $V\lambda_2$-315 failed due to a severe reduction in helper T cell responses probably caused by non-H-2 genes in the C57Bl strain. It was also found that helper T cells primed with the unfolded domains cross-reacted with the assembled folded domains of the complete myeloma protein, indicating that the antigenic site recognized by the helper cells was not dependent on conformation.

Materials and Methods

Mice. All mice were obtained from The Jackson Laboratory, Bar Harbor, ME, except for the B10.A(4R) strain, which was generously provided by Jan Klein (Max Plank Institute, Tübingen, Federal Republic of Germany), and the BALB.K strain, which was obtained from Olac 1976 (Blackthorn, Bicester, England).

Antigens. The following procedures have been described: purification of myeloma protein 315 (12), the V domains of M315 (13), conjugation of 4 hydroxy-3-iodo-5-nitro-phenylacetyl (NIP) azide to proteins (14), and iodination of NIP-caproic acid (14).

Unfolded V domains were produced by reduction of the intrachain S--S bond in 0.005 M dithiothreitol in 5 M guanidine HCl, 0.5 M Tris/HCl, pH 8.2, for 1 h at room temperature under N2 atmosphere, followed by alkylation with 0.01 M iodoacetamide for 0.5 h at 4°C and dialysis against 0.5 M guanidine HCl, 0.5 M Tris/HCl, pH 8.2. The reduction of the $V\lambda_2$ domain was complete as judged by electrophoretic analysis in acid-urea polyacrylamide gels (15); in this technique the electrophoretic mobility of a reduced (unfolded) domain is retarded relative to the unreduced form (16). The reduction of $V_{H}$-315 was also complete based on electrophoresis in sodium dodecyl sulfate-polyacrylamide (17%) gels without 2-mercaptoethanol (17). By this technique the reduced domain migrated slower than the unreduced. In addition, 10 $\mu$g of the unfolded $V\lambda_2$ did not inhibit binding of folded $^{125}$I-$V\lambda_2$ (15 ng) to BALB/c antibodies against the native free L-315, indicating that the conformational antigenic determinant recognized by this antiserum was completely absent from unfolded $V\lambda_2$.

Immunization. Priming of B cells with NIP-bovine serum albumin (BSA) (200 $\mu$g) and carrier ($V\lambda_2$ or $V_{H}$-315) priming of helper T cells was done by a single intraperitoneal injection of 100 $\mu$g antigen emulsified in complete Freund’s adjuvant (Behringwerke, containing 1 mg Mycobacterium tuberculosis cells per ml) 8 wk before cell transfer, as described (9).

Assay for Carrier-specific Helper Cell Response. Spleen cells from hapten- and carrier-primed donors were pooled, washed once, and transferred intravenously to recipients that had been x-irradiated the day before with 500 rad. Each irradiated recipient was given a total of one spleen equivalent (one-half equivalent of NIP-BSA primed and one-half equivalent of carrier-primed cells). All recipients were boosted the following day by injection of 200 $\mu$g i.p. NIP-Fab-315 in saline. It should be noted that the Fab fragment had never been in contact with denaturing solvents and was not chemically modified except for the low degree of substitution by NIP-azide. Anti-NIP antibody responses were measured 10 d after boost by a modified Farr assay as described (9).

We concluded that those animals in which a pronounced anti-NIP-antibody response was observed had received high responder T cells able to provide carrier-specific help for NIP-primed B cells, i.e., a strong carrier effect. Conversely, those animals that developed no or very low anti-NIP response, i.e., weak or absent carrier effect, were considered to have received low-responder T helper cells.

Results

Congenic Strains of Mice on the BALB/c and C3H Background. In a previous report (11) we presented evidence that $V\lambda_2$-315 elicited strong carrier effect in mouse strains of
the H-2 haplotypes d, b, and s and a low or absent carrier effect in various strains of mice of the k haplotype. A different response pattern was found for V_H-315: mice of k and s haplotype strains were high responders, whereas d and b haplotype mice were low responders. F1 hybrids produced by mating H-2^k and H-2^d parents—(C3H × BALB/c)F1 mice—were high responders to both V_H and V_L, indicating that at least for this combination the responder phenotypes were inherited in a codominant fashion. The congenic strain BALB.K, which only differs from BALB/c mice by being H-2^k instead of H-2^d, responded like k haplotype strains.

To obtain more evidence for genetic control linked to H-2, we repeated the experiments with the d and k haplotype on BALB/c background. In addition, we examined the effect of these haplotypes on C3H background. For the latter purpose the responses of the congenic C3H.H-2^o strain were compared with C3H. The H-2 haplotype of this strain arose from a recombination in the cross (C3H/Jsf X DBA/2Jsf)F1 X C3H/Jsf, and is H-2^k except at the D region, which is D^b (18). It should be noted that because all strains used in this experiment were Igh-C^a, interference by Igh-C allotypes was ruled out.

The data shown in Fig. 1 indicate that on both BALB/c and C3H backgrounds the H-2^d haplotype conferred high responsiveness to V_L-315 and low responsiveness to V_H-315. Conversely, the H-2^k haplotype conferred low responsiveness to V_L-315. On the basis of the striking differences between the responses of d and k haplotype congenic mice, we conclude that at least on these backgrounds, H-2-linked genes control helper T cell responses to the V domains of M315.

**Congenic Strains of Mice on the C57Bl/10 Background.** The effect on responsiveness of the b, d, and k haplotypes on the B10 background was studied because a series of intra-H-2-recombinant haplotypes exists on this background. The data are shown in Fig. 2, groups 1–3. The first point to be noted is that the k haplotype also conferred high responsiveness to V_H-315 on the B10 background, and the d haplotype conferred low responsiveness. This result further corroborates the H-2 linkage of the genetic differences in the responsiveness to V_H-315. Second, the absence of a carrier effect of V_H-315 in B10 mice indicates that H-2_b is a low-responder haplotype for V_H-315.

The unexpected observation was that mice of the B10.D2 congenic strain were low

| Strain | H-2 haplotype | Igh-C type | Number of recipients | Carrier priming | Secondary anti-MIP responses of recipients boosted with NIP-Fab-315 bound (\% NIP bound) |
|--------|---------------|------------|----------------------|----------------|------------------------------------------------------------------------------------------------|
| BALB/c | d             | a          | 12                   | V_L            | 0 | 20 | 40 | 60 | 80 |
| BALB/k | k             | a          | 6                    | V_L            | 0 | 20 | 40 | 60 | 80 |
| C3H    | k             | a          | 11                   | V_L            | 0 | 20 | 40 | 60 | 80 |
| C3H.H-2^o | d^* | a          | 12                   | V_L            | 0 | 20 | 40 | 60 | 80 |

**Fig. 1.** Helper cell recognition of V domains of M315 as a function of the H-2^d and H-2^k haplotypes on two different genetic backgrounds. The data express percent ± SE of 1.2 × 10^{-6} mol of NIP-caproic acid bound by 20 μl serum collected 10 d after boost.

* d region is D^b.
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| Strain       | H-2 Type | Number of Carrier recipients priming | Secondary anti-NIP responses of recipients boosted with NIP3-Fab-315 & N125ip bound |
|--------------|----------|--------------------------------------|-----------------------------------------------------------------------------------|
| B10.02       | d d d d d d d | 12                                   | Vλ2                                                                                 |
| B10.04       | k k k k k k k | 14                                   | Vλ2                                                                                 |
| B10          | b b b b b b b | 14                                   | Vλ2                                                                                 |
| B10.A(4R)    | k k k k k k d | 12                                   | Vλ2                                                                                 |
| B10.1        | k k k k k k k | 14                                   | Vλ2                                                                                 |
| B10.A(5R)    | b b b k k k d | 14                                   | Vλ2                                                                                 |

Fig. 2. Helper cell recognition of V domains of M315 as a function of various H-2 haplotypes and C57B1/10 background genes. The data are compiled from two separate and identical experiments. Not shown are the responses of BALB/c mice (84.0% for Vλ2 and 0.9% for Vμ priming). Details as in Fig. 1.

| Strain       | Number of Carrier recipients priming | Secondary anti-NIP responses of recipients boosted with NIP3-Fab-315 & N125ip bound |
|--------------|--------------------------------------|-----------------------------------------------------------------------------------|
| BALB/c       | 6                                    | MEM                                                                               |
| BALB/c       | 6                                    | folded Vλ2-315                                                                   |
| BALB/c       | 6                                    | unfolded Vλ2-315                                                                  |
| C3H          | 6                                    | MEM                                                                               |
| C3H          | 6                                    | folded Vμ-315                                                                    |
| C3H          | 5                                    | unfolded Vμ-315                                                                   |

Fig. 3. Helper cells recognize both folded and unfolded V domains of M315. Details as in Fig. 1.

responders to Vλ2, in spite of being H-2d. This is strikingly different from the high responsiveness of mice with the d haplotype on BALB/c and C3H background. The result, obtained in two separate experiments, indicates that B10 non-H-2-linked genes exert a negative regulatory effect on the T cell response to Vλ2.

B10 mice were low responders to Vλ2 (Fig. 2, group 3), which is at variance with the high responsiveness of C57Bl mice to Vλ2 reported previously (11). At the present time we are unable to explain this discrepancy, but the same result has been obtained with these strains in two separate experiments subsequent to our first report (11), and it is consistent with the lowering effect of the B10 non-H-2 genetic background described above. The observations thus far do not allow conclusions to be drawn concerning the responder status of the b haplotype for Vλ2; experiments are in
progress with congenic mice bearing the b haplotype on C3H background to clarify this issue.

Intra-H-2-recombinant Strains on the C57Bl/10 Background. To determine the map position of the immune response locus for VH-315, the helper cell responses were studied in B10.A, B10.A(4R), and B10.A(5R). As illustrated in Fig. 2 (groups 3 and 4), VH-315 elicited no carrier effect in B10 and a strong carrier effect in B10.A(4R), indicating that the presence of the high responder k haplotype in the K and I-A subregion is sufficient for an optimal helper cell response to VH-315. The high responsiveness of B10.A mice and low responsiveness of B10.A(5R) mice is consistent with this conclusion.

All six H-2 haplotypes tested on B10 background were associated with low responsiveness to VT2 (Fig. 2). This negative regulatory effect of B10 non-H-2-genes precluded further mapping of the Ir-gene for VT2.

Immunogenicity of Unfolded V Domains. More precise localization of the carrier determinants demands further fragmentation of the V domains. To examine the feasibility of such experiments and obtain information about the conformation requirements for the immunogenicity of the V regions, we studied the carrier effect of T cells primed with unfolded domains, i.e., with the intradomain disulfide bridge cleaved. The data in Fig. 3 show that the carrier effects mediated by folded and unfolded VT2 in BALB/c mice and by VH-315 in C3H mice were equivalent. This finding indicates that the helper T cells do not recognize a conformation dependent antigenic site.

Discussion

The V domains of M315 represent the first examples of Ir gene-regulated antigens that belong to Ig V domains (11). M315 affords distinct advantages for the present studies of the immunogenetics and antigenicity of V regions because of the large amount of information available about its chemistry, the paucity of V \( \lambda \) genes (6), and the ease by which its V domains can be isolated (13). The latter point made it possible to rigorously avoid T cell priming by C region antigens, which would complicate the analysis. In addition, a single V region is less antigenically complex than a complete H or L chain or an assembled pair of two different V regions, hence the probability is increased that Ir gene effects may be observed. The uniform high responsiveness of all strains tested to Fv-315 (11) indicates that the immunogenicity of one V domain masks the deficient immune response to the other. V \( \lambda \) and VH domains can thus be envisaged to complement each other's antigenicity with respect to recognition in the network. In addition, new determinants may be created by the pairing of VH-315 and V\( \lambda_p \)-315 and contribute to the antigenic heterogeneity of Fv. Consequently, the antigenic complexity of Fv may be sufficiently high to make a true low-responder haplotype for anti-Fv responses uncommon, a situation that has obvious implication for the network control of immune responses (1).

The recipients of carrier- and hapten-primed cells were boosted with hapten on Fab-315 for two reasons. First, our primary interest was directed to epitopes associated with the assembled V domains of Fab-315, because such epitopes may be more relevant for network regulation than those confined to isolated domains. Second, to our knowledge Igh-Ca allotypes have not been found in Fab\( \alpha \), decreasing the chance of allotype interference in the experiments.

The immune responses were measured as carrier effects (see Materials and Meth-
ods), a classic experimental design for monitoring helper T cell responses (19). We have previously demonstrated that the carrier effect observed with spleen cells primed with fragments and subunits of M315 is abolished by anti-Thy-1.2 plus complement treatment of the cells, demonstrating that carrier-primed T cells were required for the responses (9, 10). In contrast, the same treatment of the hapten (NIP)-BSA-primed spleen cells did not change the results (9), indicating that the T cells of the B cell donor population did not contribute to the responses. The Ir gene effects of the present report are therefore undoubtedly expressed in that T lymphocyte population which provides carrier-specific help for B lymphocytes.

The results establish that the helper cells that recognize the V domains of M315 are subject to the Ir gene defect. Thus, in contrast to H-2d, H-2k conferred high responsiveness to V_H-315 on three different genetic backgrounds, BALB/c, C3H, and B10. For V\lambda_2, H-2d was a high-responder phenotype compared with H-2k on two backgrounds, BALB/c and C3H. Thus, V_H-315 and V\lambda_2 display different Ir phenotypes. In addition, a low response phenotype for V\lambda_2 was also determined by non-H-2 genes of the B10 background which extinguished the carrier effect of V\lambda_2 in animals of the B10.D2 strain with the V\lambda_2 high-responder haplotype H-2d. Previously, B10 non-H-2 genes have been found to reduce the magnitude of the antibody response to several antigens such as GAT (20), sheep erythrocytes (21), staphylococcal nuclease (22), lysozyme (23), and collagen type V (24). In comparison, the effect of the B10 background on the helper cell responses to V\lambda_2 appeared more profound in that the carrier effect was completely abolished. Background gene effects may also influence the responses to V_H-315, because the carrier effect of V_H-315 was lower with the high responder H-2d haplotype on BALB/c than on the C3H background (Fig. 1).

Fortunately, the carrier effect of V_H-315 was not affected by the B10 background, and the low responsiveness of B10 and high responsiveness of B10.A(4R) maps the V_H-315 Ir gene to the K or I-A subregion. However, dual subregion (A plus E/C) control may also explain the data. According to this alternative, the A^kE/C^k complementation of B10.A(4R) is a high-responder phenotype in contrast to the A^kE/C^k complementation of B10.A(5R). From earlier studies, both single and dual subregion controls are known. For example, the antibody response to Igh-Ca allotypic determinants of BALB/c IgA myeloma proteins is controlled by an Ir gene in the I-A subregion (25), whereas the response to the synthetic polypeptide containing glutamic acid, lysine, and phenylalanine (GLqk) is controlled by loci that map to both I-A and I-E/C subregions (26).

The other major finding of the present study was that completely reduced and alkylated V domains primed helper cells as efficiently as native domains for responses to NIP-Fab-315. Biochemical studies have demonstrated that an intact intradomain disulfide bridge is required for proper folding of Ig domains (27, 28); in its absence the domains unfold and lose their characteristic conformation. The unimpaired immunogenicity of the unfolded domains indicates that the helper T cells recognize a conformation-independent antigenic determinant. Analogous results have been obtained by investigations of cellular immunity to conventional extrinsic protein antigens where extensive cross-reactions were observed between native and denatured forms of same antigen; in contrast, with assays that depended on conventional antibodies, no or minimal cross-reactivity was detectable (29–31). A similar dichotomy between cellular and humoral immunity to the \lambda_2-315 chain has been observed by us:
antibodies elicited in BALB/c mice by the free form of native $\lambda_2$-315 react in a sensitive double antibody radioimmunoassay with the homologous antigen, but do not bind the assembled form of the $\lambda_2$-chain in the complete M315 antibody molecule or the free form of the completely reduced and alkylated (unfolded) chain (see Materials and Methods). Evidently, humoral immunity to $\lambda_2$-315 recognizes a conformation-dependent antigenic site that is hidden in the complete M315.

The carrier-specific helper cells of the present study should be compared with a new kind of T helper cell specific for determinants that seem to resemble serologically defined idiotypes (32–36). These T cells act in combination with conventional carrier-specific helper cells, but are distinct from the latter by their idotype specificity and by their ability to deliver help to B cells independent of a hapten-carrier bridge. Their presence is detected by their ability to promote certain B lymphocytes bearing a given public idotype to mature into antibody-secreting cells. In some instances, these idotype-specific helpers have been depleted by incubation on plastic dishes coated with antibodies bearing the idotype (32, 34), and in one system they are present before immunization as natural helper cells (36) and their interaction with B cells bearing the complementary idotype is not MHC restricted (37). The fine specificity of this set of T helper cells has, however, not been defined. In contrast, the helper cells of the present study are not detectable before priming, and they resemble conventional helper cells by their performance in the context of a hapten-carrier bridge, the Ir gene control of their responses and their lack of discrimination between native and unfolded polypeptides. No information is yet available about MHC-restriction.

We do not yet know whether the V domain-specific helpers function in the network. One can envisage at least two mechanisms for such a function. The first is analogous to a hapten-carrier bridge. According to this idea, circulating antibodies bearing $V_{H}$-315 or $V\lambda_2$-like V domains bind to B cells with complementary (anti-idiotypic) Ig receptors. These B cells could receive help from T cells of a kind described in this report across a bridge formed by the bound antibody; the result should be an augmented anti-idiotypic antibody response. The idotype recognized by these B cells could be very different from that of M315, since it is probable that $V\lambda_2$ and $V_{H}$-315-like domains have the potential to associate with many different $V_H$- and $V_L$-domains, respectively. The second mechanism is visualized as direct delivery of help to B cells that synthesize Ig receptors with $V\lambda_2$- or $V_{H}$-315-like domains. This help differs from the first in that it does not require a hapten-carrier bridge, but it would probably require antigen stimulation of the B cells to make them receptive to helper signals. In this manner, $V\lambda_2$-specific helper cells would promote synthesis of specific antibodies bearing $\lambda_2$-chains.

Summary

We have examined the recognition of the variable (V) domain of the heavy ($V_H$) and light ($V\lambda_2$) chains of mouse myeloma protein 315 by helper T cells. Mice were primed with the isolated V domain in complete Freund’s adjuvant, and carrier (V domain)-primed spleen cells were transferred together with hapten (NIP)-primed spleen cells to recipient mice that were boosted with NIP-Fab-315. The helper cell response to both domains was governed by H-2-linked immune response (Ir) genes, and $V_{H}$-315 and $V\lambda_2$ displayed different Ir phenotypes. H-2$^k$ conferred high responsiveness to $V_H$ on three different genetic backgrounds, BALB/c, C3H, and B10; mice
of the d and b haplotypes were low responders. Conversely, H-2d conferred high responsiveness to V\textsubscript{d} on two backgrounds, BALB/c and C3H, whereas mice of the k haplotype were low responders to this domain. Non-H-2 genes of the B10 background extinguished the helper cell response to V\textsubscript{d} in animals with the high responder d haplotype. The V\textsubscript{H}Ir gene mapped to the K-A interval of the H-2 complex. Unfolded (completely reduced and alkylated) V domains primed helper cells as efficiently as folded domains for responses to NIP\textsubscript{a}-Fab-315, indicating that the helper cells recognized an antigenic determinant that was not conformation-dependent. The data indicate that there exists helper T cells which recognize each member of the M315 pair of V domains independent of the other, and that these V domains are recognized like conventional extrinsic protein antigens.

We thank Anne Store for excellent technical assistance and Henny Johansen for typing the manuscript.

Received for publication 28 December 1981.

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