Brief Definitive Report

EXPRESSION OF A SINGLE MAJOR HISTOCOMPATIBILITY COMPLEX LOCUS CONTROLS THE IMMUNE RESPONSE TO POLY-L-(TYROSINE, GLUTAMIC ACID)-POLY-DL-ALANINE--POLY-L-LYSINE*

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Genetic control of the immune response linked to the major histocompatibility (*H-2*) complex in the mouse has been described for synthetic polypeptide antigens and for low doses of native proteins. The phenomenon is well documented (1, 2). Extensive screening of intra-*H-2* crossover-derived recombinant strains has localized *H-2*-linked immune response (*Ir*) genes to the *I*-immune response region of the *H-2* complex (3). For most antigens, *Ir* genes are autosomal, dominant, and they segregate as single loci. It is not known whether these crossover-defined loci represent single genes with multiple alleles or clusters of tightly linked genes (4).

In 1972, Stimpfling and Durham (5) postulated that two interacting loci within the *H-2* complex were required for the response to the alloantigen, H-2.2 (6), and, in 1975, Dorf et al. (7) observed a responder phenotype in a recombinant derived from two strains which were nonresponders to the synthetic linear terpolymer, L-glutamic acid, L-lysine, L-phenylalanine (GLPhe). Analysis of additional recombinants and complementation tests with F_1 hybrids clearly demonstrated that genes in two intra-*I*-region loci controlled the immune response to GLPhe. Subsequently, requirements for genes mapping in two intra-*I*-region loci were reported for porcine LDH_B (8), the alloantigen Thy-1.1 (9), and for the synthetic terpolymers L-glutamic acid, L-lysine, L-tyrosine and L-glutamic acid, L-lysine, L-leucine (6, 10).

Demonstration that responses to both synthetic polypeptide and native protein antigens can be controlled by genes in two distinct *I*-region loci prompted speculation that the phenotypic expression of two *I*-region genes is a general phenomenon which may provide the key for understanding the mechanism of *Ir* gene function and cellular collaboration in the immune response. Benacerraf and Dorf (10) have shown that *Ir* gene complementation is often more effective in the *cis* than in the *trans* configuration. This concept is further supported by the data reported for GLPhe (10–12) which indicate that both of the complementing genes must be expressed in each of the cell types participating in the interaction. Failure to detect complementation for the majority of antigens under *H-2*-linked *Ir*-gene control might be attributed to the limited number of available intra-*I*-region recombinant strains.

Munro and Taussig (13) reported *trans* complementation for the primary IgM response

* Supported in part by National Institutes of Health grant AI 07757, and contract CB 43941.

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to the synthetic random polypeptide poly-L-(tyrosine, glutamic acid)-poly-DL-alanine--poly-L-lysine, abbreviated (T, G)-A--L, both in vivo in (B10.M × B10.BR)F_1 (*H-2*^{f} × *H-2*^{k}) hybrids, and in a thymus-derived helper factor-bone marrow reconstitution system in which B10.BR factor complemented B10.M bone marrow. The results of the reconstitution experiments suggested that the complementing genes are expressed in distinct lymphocyte populations. The complemented response was equivalent to that of the responder phenotype (*H-2*^{b}) in both systems.

Complementation for the response to (T, G)-A--L has also been reported by Swartz et al. (12). Using an antigen-induced T- (thymus-derived) lymphocyte proliferation assay, intermediate levels of stimulation between low- and high-responder phenotypes were obtained in the (B10.M × B10.BR)F_1 hybrid. The T-lymphocyte proliferation assay is reported to be B- (bone marrow-derived) lymphocyte independent (12). These findings suggest, therefore, that coexpression of both genes must occur in another cell population; presumably in T cells and/or macrophages.

Extensive screening in our laboratory of the IgG secondary response to (T, G)-A--L of intra-*H-2* crossover-derived recombinant strains (3), and of F_1 hybrids derived from low-responder parents (B. Deak and H. O. McDevitt, unpublished observations) did not suggest a requirement for two separable *I*-region genes. However, given the limited numbers of recombinant strains available for analysis and the demonstrated two-gene requirement in several systems, additional F_1 hybrids were studied for both primary and secondary in vivo response to (T, G)-A--L.

Materials and Methods

A minimum of six mice per group were primed intraperitoneally with 10 μg (T, G)-A--L (batches 52 or 1383) in complete Freund's adjuvant. These mice were bled on days 14 and/or 21 for the primary response, boosted intraperitoneally on day 21 with 10 μg antigen in phosphate-buffered saline, and bled again on day 28 for the secondary response. Each experiment included a group of animals possessing the *H-2*^{b} (high responder) haplotype.

Levels of IgM and IgG antibodies were determined by an assay for antibody-forming cells (plaque assay) (14), by the binding of serum antibodies to antigens in solution (modified Farr assay) (3), and by the binding of serum antibodies to antigens fixed in the solid phase in microtiter plates (plate assay) (15).

Results

The results of immunizing multiple combinations of F_1 hybrids derived from crosses between low-responder haplotypes are shown in Fig. 1, in which all F_1 crosses are between *H-2* congenic partners, and in Fig. 2, in which all F_1 crosses are noncongenic. None of the F_1 hybrids produced a primary or secondary response greater than that of the parental strains, including (B10.M × B10.BR)F_1, which was reported by Munro and Taussig (13) and Swartz et al. (12) to complement to produce a high response.

The secondary response of strain A.CA (Fig. 2) is intermediate, and is most likely the result of the influence of non-*H-2* genes on the magnitude of the response. A comparison of the high-responder allele, *H-2*^{b}, on the B10 and A backgrounds (16) clearly demonstrates this effect. F_1 hybrids between A.CA and low-responder haplotypes respond to a lesser degree than does A.CA (Fig. 2), providing additional evidence for lack of complementation.

To resolve this discrepancy, B10.BR, B10.M, and (B10.M × B10.BR)F_1 mice, kindly supplied by Dr. Alan Munro (Cambridge University, Cambridge, Eng-

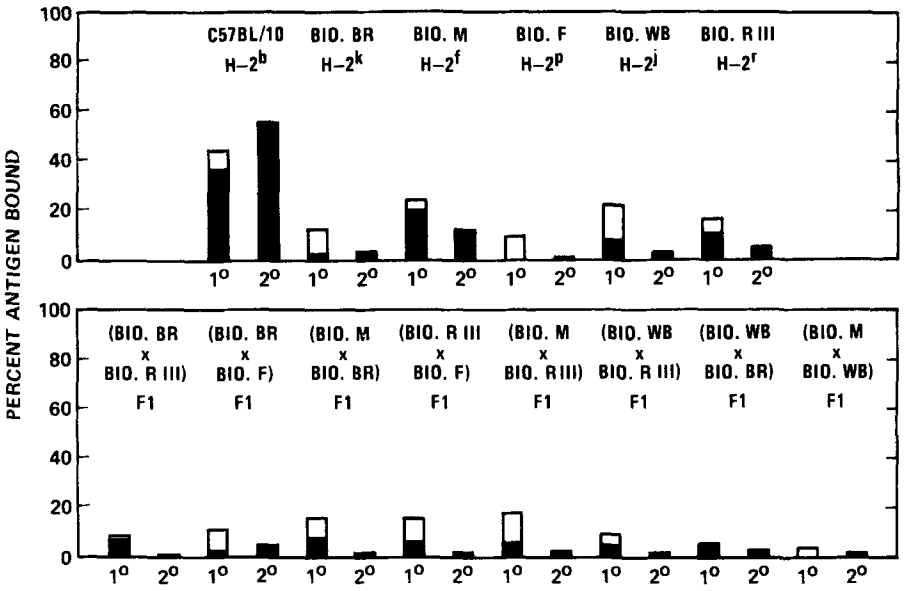

FIG. 1. Primary and secondary response to 10 μg (T, G)-A--L 52 in congenic F_1 hybrids, determined by binding of pooled serum antibodies to ^{125}I-labeled (T, G)-A--L, in a modified Farr assay. The sera were assayed at a dilution of 1:50 for the primary (1°) response and 1:250 for the secondary (2°) response. The shaded areas represent 2-mercaptoethanol-resistant antibodies, which are assumed to be IgG. IgM antibodies are total antibodies minus 2-mercaptoethanol-resistant antibodies.

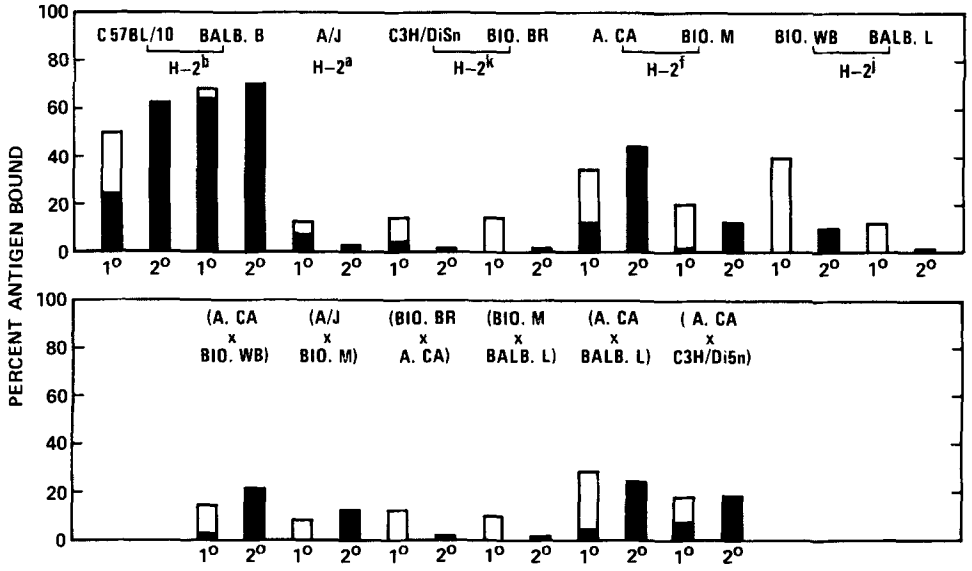

FIG. 2. Primary and secondary response to 10 μg (T, G)-A--L 1383 in noncongenic F_1 hybrids by binding of pooled serum antibodies to ^{125}I-labeled (T, G)-A--L in a modified Farr assay. The sera were assayed at a dilution of 1:50 for the primary (1°) response and 1:250 for the secondary (2°) response. The shaded areas represent 2-mercapthoethanol-resistant antibodies, which are assumed to be IgG. IgM antibodies are total antibodies minus the 2-mercaptoethanol-resistant antibodies.

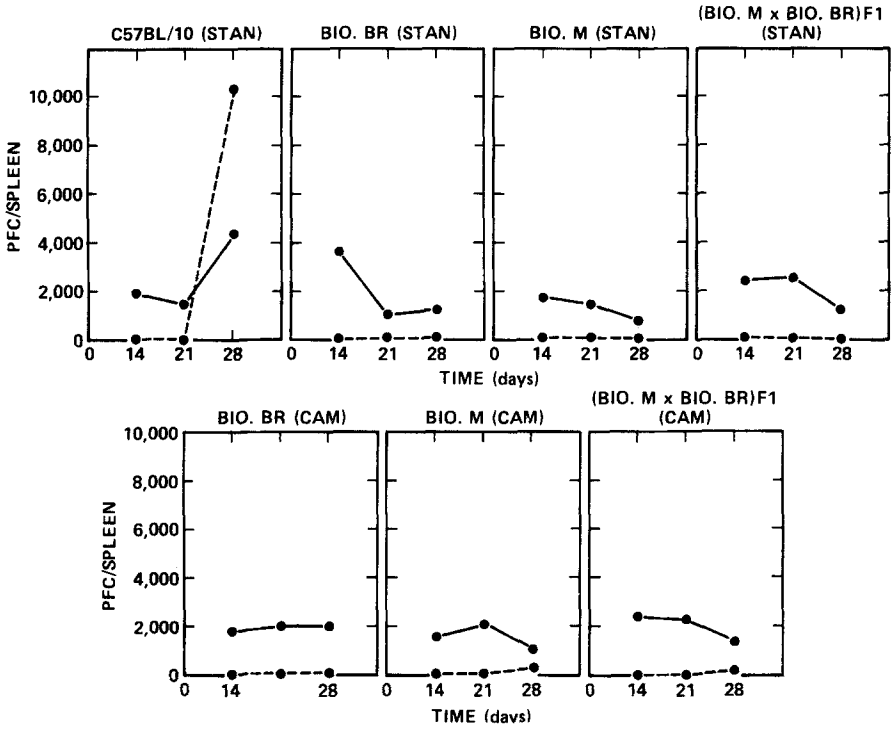

FIG. 3. Comparison of the primary and secondary antibody-forming cell response to 10 μg. (T, G)-A--L 1383 in Stanford (Stan) lines and Cambridge (CAM) lines. The direct (–) number of plaque-forming cells (PFC) was determined with poly-L-(tyrosine, glutamic acid)-poly-DL-proline--poly-L-lysine-tagged sheep erythrocytes. The indirect (---) PFC number was determined with (T, G)-A--L-tagged sheep erythrocytes.

land), were used in concert with B10, B10.BR, B10.M, and (B10.M × B10.BR)F_1 mice bred in our facilities at Stanford. The mice were grouped so that parallel determinations of the antibody response by plaque assay, modified Farr assay, and plate assay could be determined on days 0, 14, 21, and 28. The results of these determinants by three independent methods were concordant. The results for the plaque assay are presented in Fig. 3. The results for the modified Farr assay and the plate assay are not shown. None of the assay methods detected complementation in the F_1 for high responsiveness in the primary or the secondary response.

A similar study using the related random synthetic polypeptide antigen poly-L-(histidine, glutamic adic)-poly-DL-alanine--poly-L-lysine produced analogous results (data not shown).

Discussion

The differences observed between phenotypes in the (T, G)-A--L system are quantitative, and it is the level of responsiveness that is under genetic control.

Variations in response patterns to different preparations of (T, G)-A--L are not uncommon in the primary response (17), and, for some but not all preparations, high and low responders are equivalent (2). Variations in secondary IgG response patterns to different (T, G)-A--L preparations have not been

observed (B. Deak and H. O. McDevitt, unpublished observations).

This quantitative difference is most obvious in the secondary IgG response. Clearly, there is significant variation in the level of response among strains of low-responder phenotype, particularly for the IgM primary. Amplification by non-*H-2* genes of the IgG secondary response has been observed for some low-responder haplotypes.

While two-gene complementation in the genetic control of the immune response to other antigens certainly exists (7), we cannot confirm a similar complementation in the immune response to (T, G)-A--L. These results clearly show that there is no in vivo complementation in F_1 hybrids derived from low-responder parents for the IgM primary or the IgG secondary immune response.

Reported in vivo complementation (13) in the primary immune response to (T, G)-A--L in (B10.M × B10.BR)F_1 hybrids has been cited as an important confirmation of earlier results (18, 19) which indicated that genes in the *I-A* subregion controlled the production of a T-cell factor and a B-cell acceptor, both of which were specific for (T, G)-A--L and were required for the production of a high response. Despite repeated attempts, we have found these results difficult to reproduce (D. Meruelo and H. O. McDevitt, unpublished observations).

Our failure to confirm the reported in vivo complementation in the (B10.M × B10.BR)F_1 hybrid in repeated experiments employing several different antibody assays, or to demonstrate complementation in multiple combinations of low responder F_1 hybrids, indicates that the proposed "factor-acceptor" model lacks confirmation by an independent experimental approach. In the absence of such confirmation, we feel this model for *Ir* gene expression cannot be considered to be established.

The authors wish to thank Dr. Jack H. Stimpfling for his generous gift of mice.

Received for publication 25 October 1977.

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