Brief Definitive Report

Mls IS NOT A SINGLE GENE, ALLELIC SYSTEM
Different Stimulatory Mls Determinants Are the Products of at Least
Two Nonallelic, Unlinked Genes

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The set of cell surface determinants encoded by the MHC is remarkable in that it is recognized by naive T cell populations at a sufficiently high precursor frequency to induce substantial primary proliferative responses, and the critical biologic role played by MHC products has been well established. The only other determinants capable of inducing strong proliferative responses by naive T cells have been identified in the mouse as minor lymphocyte stimulating (Mls) gene products (1, 2). It was originally proposed by Festenstein that Mls is a single gene system mapped to chromosome 1 (3), and that the Mls locus has at least four alleles, a, b, c, and d (1), which encode polymorphic cell surface structures on B cells and macrophages. Subsequent studies have documented the extraordinarily high frequency of Mlsa- or Mlsd-reactive T cells encountered in both naive (2) and cloned T cell populations (4).

In spite of its initial characterization, the issues of polymorphism and allelism in the Mls system are matters of significant controversy. Because of a high degree of observed crossreactivity between Mlsa and Mlsd determinants (5–7) and the nonexistent or weak stimulatory ability of Mlsb and Mls` stimulators, some investigators (7) have suggested that the Mls locus has only two alleles, the “a/d” allele encoding nonpolymorphic determinants which cause strong MLR, and the nonstimulatory or null “b” allele. Based on this interpretation, it has recently been proposed that the Mls gene encodes molecules that cause nonspecific mitogenic stimulation to T cells (7) or that Mls products are nonpolymorphic cell interaction structures that are of generalized importance in influencing the interaction between APCs and responding T cells (8). In an attempt to better define polymorphism and allelism in the Mls system, studies were initiated using cloned T cells specific for each of the stimulatory Mls alleles. These studies demonstrated that Mlsa and Mlsd determinants as recognized by cloned T cells are reciprocally non-crossreactive, indicating that polymorphism does indeed exist in the Mls system (9). More strikingly, it was also found that Mlsd strains coexpress both Mlsa-like and Mlsd-like determinants, and that these two sets of determinants are encoded by unlinked genes in the Mlsd genome (10). These
findings suggested the possibility that the genes encoding Mls\textsuperscript{a} and Mls\textsuperscript{b} were themselves nonallelic, and that the original description of Mls\textsuperscript{a}, Mls\textsuperscript{b}, and Mls\textsuperscript{c} as allelic products of a single locus was not correct. To formally analyze the genetic characteristics of the Mls gene system, a segregation analysis of genes encoding Mls\textsuperscript{a} and Mls\textsuperscript{b} determinants was carried out.

**Materials and Methods**

**Mice.** AKR/J, B10.BR, and C3H/HeJ mice were purchased from The Jackson Laboratory, Bar Harbor, ME. (AKR/J × B10.BR)\textsubscript{F\textsmaller{1}}, (AKR/J × C3H/HeJ)\textsubscript{F\textsmaller{1}}, and (AKR/J × C3H/HeJ) × B10.BR mice were bred in our facilities.

**Antibody.** Goat anti-mouse IgD antibody was kindly provided by Dr. Fred D. Finkelman (Uniformed Services University of the Health Sciences, Bethesda, MD). The use of this antibody has been described elsewhere (9–11).

**T Cell Clones.** The generation and characterization of Mls\textsuperscript{a}, Mls\textsuperscript{b}, and I-A\textsuperscript{a}-reactive clones have been described (9).

**T Cell Proliferation Assay.** T cell proliferation was assayed as previously described (9, 10). Results are expressed as the arithmetic means of triplicate cultures. Standard errors were generally <10% of the mean.

**Results**

**Segregation Analysis of Genes Encoding Mls\textsuperscript{a} and Mls\textsuperscript{b} Determinants.** To evaluate the allelic or nonallelic nature of the genes encoding Mls\textsuperscript{a} and Mls\textsuperscript{b} determinants, a segregation analysis was carried out by testing the stimulatory capacity of spleen cells from progeny of the (AKR/J × C3H/HeJ)\textsubscript{F\textsmaller{1}} × B10.BR [(Mls\textsuperscript{a} × Mls\textsuperscript{b})\textsubscript{F\textsmaller{1}} × Mls\textsuperscript{b}] breeding. The hypothetical basis of this segregation analysis was the following. If the Mls\textsuperscript{a} and Mls\textsuperscript{b} determinants were encoded by allelic genes, all of the offspring from this breeding would be either Mlsa/b or Mls\textsuperscript{b} genotypically. Since Mls\textsuperscript{b} is nonstimulatory, there would be phenotypically only two types of stimulator cells, one of these Mls\textsuperscript{a} and the other Mls\textsuperscript{b}. Alternatively, if the genes encoding Mls\textsuperscript{a} and Mls\textsuperscript{b} were not allelic, then stimulator cells from some progeny might express both Mls\textsuperscript{a} and Mls\textsuperscript{b} or neither Mls\textsuperscript{a} nor Mls\textsuperscript{b}. A total of 70 progeny from the (AKR/J × C3H/HeJ)\textsubscript{F\textsmaller{1}} × B10.BR breeding were tested in five different experiments. In the first experiment presented here, mitomycin C (MMC)-inactivated spleen cells from these progeny were used as stimulators and were cocultured with unprimed C3H/HeJ (Mls\textsuperscript{b}) nylon wool nonadherent T cells (NNT) to detect Mls\textsuperscript{a} determinants, with (AKR/J × B10.BR)\textsubscript{F\textsmaller{1}} (Mls\textsuperscript{a/b}) NNT to detect Mls\textsuperscript{b} determinants, or with cloned I-A\textsuperscript{a}-specific T cells to test the overall stimulatory abilities of each stimulator cell population (Table I). Spleen cells from all 10 progeny stimulated the I-A\textsuperscript{a}-specific T cell clone. Stimulator cells from some of these progeny stimulated C3H/HeJ (Mls\textsuperscript{a}) NNT, but not (AKR/J × B10.BR)\textsubscript{F\textsmaller{1}} (Mls\textsuperscript{a/b}) NNT (Nos. 5, 6), indicating that these cells express Mls\textsuperscript{a} but not Mls\textsuperscript{b} (consistent with an Mls\textsuperscript{a/b} genotype); whereas cells from other progeny stimulated only NNT from (AKR/J × B10.BR)\textsubscript{F\textsmaller{1}}, but not C3H/HeJ (Nos. 4, 10), indicating that they expressed Mls\textsuperscript{a} but not Mls\textsuperscript{b} (consistent with an Mls\textsuperscript{a/b} genotype). In addition, however, some offspring populations stimulated both C3H/HeJ and (AKR/J × B10.BR)\textsubscript{F\textsmaller{1}}, NNT (Nos. 2, 3, and 8), indicating coexpression of Mls\textsuperscript{a} and Mls\textsuperscript{b} by these stimulators; and others stimulated neither of these T cell populations (Nos. 1, 7, and 9), indicating expression of neither Mls\textsuperscript{a} nor Mls\textsuperscript{b}.
This analysis was extended by two additional approaches. In the first of these, the magnitude of primary Mls<sup>a</sup> responses was enhanced by using stimulator cells from mice which had been treated in vivo with anti-IgD antibody (11). When assayed for their ability to stimulate NNT from CSH/Hej (Mls<sup>c</sup>) or AKR/J (Mls<sup>b</sup>)
mice in order to detect Mlsα and Mlsβ expression, respectively, (AKR/J × C3H/HeJ)F1 × B10.BR offspring again revealed phenotypically 4 different patterns of Mls expression: Mlsα type (No. 12), Mlsβ type (Nos. 11, 13, 15, and 18), Mlsαβ type (Nos. 17, 19, and 20), and Mlsα-negative, Mlsβ-negative (Nos. 14, 16) (Table II). In another experiment, the Mls type of additional progeny was examined by using Mlsα- and Mlsβ-specific T cell clones (Table III) (9). 12 offspring mice were tested and again separated into four groups: Mlsα-type (Nos. 27, 29); Mlsβ-type (Nos. 21, 23, 25, 31, and 32); Mlsαβ-type (Nos. 22, 24, and 26); and Mlsα-negative, Mlsβ-negative (Nos. 28, 30). Of the total of 70 progeny tested in all experiments, 37 exhibited either coexpression or nonexpression of both Mlsα and Mlsβ determinants, indicating that these two sets of determinants are encoded by distinct and unlinked genes.

Discussion

The issues of allelism and polymorphism are of fundamental importance to an understanding of the biology of the Mls system. If Mls is interpreted, as has conventionally been the case, as a single locus, multi-allelic polymorphic system, then attempts to understand the nature of T cell recognition of Mls may center on mechanisms involving a polymorphic T cell recognition structure (such as the conventional T cell receptor α/β dimer). In contrast, if the character of Mls products is found to differ from this conventional model, then the nature of the relevant TCR structures for Mls may also differ. We have recently demonstrated (9) that Mlsα-specific clones respond to Mlsα but not Mlsβ, while Mlsβ-specific clones respond to Mlsβ but not Mlsα, a reciprocal pattern of specificity indicating that polymorphism does exist between Mlsα and Mlsβ. Surprisingly, however, it
was also found (10) that both Mlsa-specific and Mls' specific clones were responsive to Mlsd strain stimulators, indicating that Mlsa strains expressed Mlsa-like as well as Mls' like determinants. A genetic analysis further demonstrated that the Mlsa-like and Mls' like determinants expressed by Mlsa cells were encoded by nonallelic genes (10). These studies raised the possibility that the prototypic Mlsa and Mls' products themselves, which were initially described as allelic products of a single locus, might be encoded by independent gene loci. The present study, using monospecific T cell clones to Mlsa and Mls', in combination with primary anti-Mls responses, has indicated by formal segregation analysis that the genes encoding Mlsa and Mls' determinants are neither allelic nor closely linked. In association with recent findings that indicated that anti-Mlsa T cell responses are in fact composed of clonally distinct responses to antigenically noncrossreactive Mlsa and Mls' determinants (10, 12), the present results suggest that Mlsa does not represent an independent genotype, but rather the coexpression in Mlsa strains of the products of unlinked Mlsa and Mls' genes.

The findings presented here provide a genetic characterization of the Mlsa system that is substantially different from the conventional model. What were originally defined as Mlsa-specific T cell responses now appear to consist of responses to determinants controlled by at least two independent gene loci. One set of these determinants is controlled by a gene located on the Mlsa locus mapped to chromosome 1, and this gene product (originally designated Mlsa) is expressed on Mlsa- and Mlsd-type cells. Another, controlled by a gene that has not yet been mapped and the products of which have been designated Mls', is expressed on Mls' and Mlsd-type cells. Since Mlsb is nonstimulatory for T cells in most response systems, there may be no polymorphism within either the Mlsa or the Mls' system. Because Mls appears to consist of relatively or absolutely nonpolymorphic products of each of two (or more) unlinked genes, the strong stimulatory capacity encoded by Mlsa genes may be mediated by interactions with T cell structures that are themselves nonpolymorphic, consistent with the previous proposals of Webb et al. (7) and Janeway et al. (8). It remains to be determined whether the determinants encoded by Mlsa and Mls' are distinct functionally as well as genetically and what role they play in the generation and expression of the antigen-specific T cell repertoire.

Summary

Mlsa determinants share with MHC products the unique property of stimulating T cells at extraordinarily high precursor frequencies. The Mlsa system was originally described as a single locus on chromosome 1, with four alleles, Mlsa, Mlsb, Mls', and Mlsd, that encode polymorphic cell surface structures. However, the fundamental issues of polymorphism and allelism in the Mlsa system remain controversial. To clarify these questions, a formal segregation analysis of the genes encoding Mlsa and Mls' determinants was carried out by testing the capacity of spleen cells from progeny of (Mlsa x Mls')F1 x Mlsb breedings to stimulate responses by unprimed T cells and by Mlsa- and Mls' specific cloned T cells. The results of this analysis indicated that the gene encoding Mlsa determinants is neither allelic to nor linked to the gene encoding Mls' determinants. Together with previous findings (12), these results also suggest that another strongly
stimulatory type, Mls^d, in fact results from the independent expression of unlinked Mls^a and Mls^c gene products. Based on these observations, it is concluded that, contrary to conventional concepts, the stimulatory phenotypes designated as Mls^a, Mls^c, and Mls^d can be accounted for by the independent expression of the products of at least two unlinked gene loci.

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References

1. Festenstein, H. 1973. Immunogenetic and biological aspects of in vitro lymphocyte allotransformation (MLR) in the mouse. Transplant Rev. 15:62.
2. Janeway, C. A., Jr., E. A. Lerner, J. M. Jason, and B. Jones. 1980. T lymphocytes responding to Mls-locus antigens are Lyt-1^+,2^- and I-A restricted. Immunogenetics. 10:481.
3. Festenstein, H., C. Bishop, and B. A. Taylor. 1977. Location of Ms locus on mouse chromosome 1. Immunogenetics. 5:357.
4. Lynch, D. H., R. E. Gress, B. W. Needleman, S. A. Rosenberg, and R. J. Hodes. 1985. T cell response to Mls determinants are restricted by crossreactive MHC determinants. J. Immunol. 134:2071.
5. Ryan, J. J., A. Ahmed, P. Kind, and K. W. Sell. 1979. Crossreactivity exists between Mls^a and Mls^d lymphocyte-activating determinants as demonstrated by the negative clonal selection of responder cells in a mixed lymphocyte reaction. Transplant. Proc. 9:1377.
6. Molnar-Kimber, K. L., and J. Sprent. 1981. Evidence that strong Ms determinants are nonpolymorphic. Transplantation (Baltimore). 31:376.
7. Webb, S. R., K. Molnar-Kimber, B. Jennifer, J. Sprent, and D. B. Wilson. 1981. T cell clones with dual specificity for Ms and various major histocompatibility complex determinants. J. Exp. Med. 154:1970.
8. Janeway, C. A., Jr., P. J. Conrad, J. Tite, B. Jones, and D. B. Murphy. 1983. Efficiency of antigen presentation differs in mice differing at the Ms-locus. Nature (Lond.). 306:80.
9. Abe, R., J. J. Ryan, F. D. Finkelman, and R. J. Hodes. 1987. T cell recognition of Ms: T cell clones demonstrate polymorphism between Ms^a, Ms^c and Ms^d. J. Immunol. 138:373.
10. Abe, R., J. J. Ryan, and R. J. Hodes. 1987. Clonal analysis of the Ms system. A reappraisal of polymorphism and allelism among Ms^a, Ms^c, and Ms^d. J. Exp. Med. 165:1113.
11. Ryan, J. J., J. J. Mond, F. D. Finkelman, and I. Scher. 1983. Enhancement of the mixed lymphocyte reaction by in vivo treatment of stimulator spleen cells with anti-IgD antibody. J. Immunol. 130:2534.
12. Ryan, J. J., J. J. Mond, and F. D. Finkelman. 1987. The Ms^d-defined primary mixed lymphocyte reaction: a composite response to Ms^a and Ms^c determinants. J. Immunol. 138:4085.