ANALYSIS OF THE IMMUNOLOGICAL MECHANISMS IN THE F₁ HYBRID ANTI-PARENTAL REACTIVITY, AND DETECTION OF A NEW MINOR HISTOCOMPATIBILITY 42(H-42) LOCUS BY F₁ CYTOTOXIC T LYMPHOCYTES GENERATED UNDER THE CONDITION OF GRAFT-VERSUS-HOST REACTION

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ABSTRACT

Lethally X-irradiated F₁ hybrid mice of some strains do not accept bone marrow transplants from one or another of the parental strains, and normal un-irradiated F₁ mice often exert resistance against the graft-vs-host reaction (GvHR)-associated immunosuppression induced by lymphocytes from a certain parental strain. Moreover, normal F₁ mouse spleen cells can respond to parental antigen coded for by the major histocompatibility complex (MHC) and generate cytotoxic T lymphocytes, which specifically kill target cells bearing the homozygous parental MHC antigens, in primary in vitro cultures. All of these phenomena are apparently a violation of the basic law of classical transplantation immunity in which the co-dominant phenotypic expression of histocompatibility gene products in F₁ hybrid animals has been established. Therefore, the study on the mechanisms of these phenomena, called hybrid resistance (HyR), embraces many important yet unexplained aspects of transplantation immunity, and offers current immunology an intriguing issue to be explored.

To explore complex genetic events that lead to HyR at cellular level, we have studied in detail the in vitro primary F₁ anti-parental cytotoxic responses as well as the F₁ resistance against parental lymphocyte-induced GvHR by using various kinds of inbred mouse strains carrying well-characterized genetic background. The regulation of both types of the HyR was found to be controlled in appearance by genes located either in the MHC or non-MHC regions. However, our careful experiments have demonstrated that, in most cases, neither the MHC haplotype alone nor the non-MHC background alone can foretell whether a certain F₁ mouse strain exerts the HyR or not. In a certain F₁ mouse strain, it
has been demonstrated that conspicuous combined action of the MHC and non-MHC genes determines the HyR. Implications of this key observation with respect to the immunologic significance of the HyR, together with the discovery of a new mouse minor H-42 histocompatibility locus, are described in detail in the present review article.

Key words: F1 hybrid anti-parental resistance, Hemopoietic histocompatibility, F1 anti-parental CTL, Graft-versus-host reaction, Minor H-42 locus

1. INTRODUCTION

It is a general rule that gene products of the histocompatibility (H) loci including the major histocompatibility complex (MHC) are co-dominantly expressed. Thus, F1 hybrid animals raised by intercrossing two inbred strains accept skin-as well as tissue-transplants from either of their parental inbred strains. For the past 3 decades, however, transplantation immunologists have noticed repeatedly the intriguing fact that some lined lymphoma cells of parental A strain origin grew deficiently in (A × B)F1 hybrid mice,1-3 and also the fact that bone marrow (BM) transplants from parental A mouse strain could not proliferate in heavily irradiated (A × B)F1 mice compared with the transplants in syngeneic recipients.4-6 According to the aforementioned transplantation rule, lymphoid cell populations from the parental A and B mice would be expected to have the same ability to mount a graft-vs-host reaction (GvHR) being associated with the suppression of immune responsiveness in the (A × B)F1 mice.7-16 This is not, however, universally observed.7,10-13,15,16 For instance, injection of parental H-2b (H-2; mouse MHC) lymphocytes into H-2k/b or H-2d/b F1 mice resulted in GvHR status, rendering striking suppression of subsequent in vitro cytotoxic T lymphocyte (CTL) responses of such F1 spleen cells to third-party alloantigens or trinitrophenyl (TNP)-modified self lymphocytes,11 whereas the F1 spleen cells in GvHR induced by parental H-2k or H-2d lymphocytes retained full ability to elicit the in vitro CTL responses.11-17,16 More interestingly, in vitro observation reminiscent of the in vivo F1 reactivities against parental lymphomyeloid transplants had been first made by Shearer and Pollison,17 and was subsequently confirmed by ourselves.18-22 In these studies, normal lymphoid cells from F1 mice developed CTL when cultured with X-irradiated or mitomycin C-treated parental spleen cells, and these CTL specifically lysed parental targets bearing homozygous H-2 antigens but not the heterozygous F1 target cells.

Thus, these phenomena of F1 reactivity to and, hence, recognition of parental antigens expressed on lymphomyeloid grafts so far described are apparently a violation of the classical transplantation immunity and have been collectively called as “hybrid resistance (HyR).”11-25

In this review article, I will address the recent progress that has been made during the past two decades in immunobiology of the HyR, and also, would like to deal with our own experimental observations which, I believe, could contribute to better under-
standing of an apparently complex phenomenon called as HyR in a general term.

2. PRIMARY IN VITRO CYTOTOXIC RESPONSE OF F₁ T LYMPHOCYTES AGAINST PARENTAL ANTIGENS

The term HyR has been used to describe the resistance of F₁ mice against BM or certain tumor cell transplants from one or another of the parental strains. An analysis of the genes controlling the parental antigens has led to the description of a number of noncodominantly expressed hemopoietic histocompatibility (Hh) loci, some of which are linked to the mouse MHC (H-2). For example, Hh-1 has not been dissociated from H-2D, nor Hh-3 dissociated from H-2K. The failure of Hh-incompatible BM grafts in heavily irradiated F₁ mice has been shown to be attributable to resistance by the F₁ host and is considered to have an immunological basis by number of criteria. Nevertheless, the immunological basis of the rejection of the hemopoietic cell graft and the mechanisms responsible for such reactivity could be more clearly understood if an in vitro model, similar to that employed for CTL responses, was available for analysis of the Hh system.

A possible in vitro correlate of the HyR has been seen in an in vitro cytotoxic model studied by several investigators. In these studies, normal lymphoid cells from F₁ mice developed CTL when cultured for 5-days with x-irradiated or mitomycin C-treated parental stimulator spleen cells. The resultant CTL specifically killed parental but not F₁ target cells, and the target antigens appeared to be those defined as Hh-I and Hh-3. Thus, in most cases the target specificities of these in vitro F₁ anti-parental CTL were mapped in either the H-2K or H-2D region of the mouse MHC, but not simultaneously in both, and the CTL were able to lyse lymphoid tumors, peritoneal exudate cells (PEC) or mitogen-stimulated lymphoid cells from parental stimulator origin but not those from responder F₁, the other parent or allogeneic strains. (C57BL/6 × DBA/2)F₁ (H-2b/d) anti-C57BL/6 CTL kill target cells homozygous at H-2Db (Hh-1) but not H-2Kb, whereas (B10.BR × 129)F₁ (H-2b/b) anti-B10.BR CTL and (AKR/J × BALB/c)F₁ (H-2k/d) anti-AKR/J CTL kill target cells homozygous at H-2Kk (Hh-3) but not H-2Dk, irrespective of the non-MHC general genetic background. Although the target antigens can be mapped in the MHC region, this may only be an indication of the position of the MHC restriction antigen. The actual target antigen(s) and the reason for the preference for H-2K or H-2D is unclear.

It is well established that CTL are generated to allogeneic MHC antigens and also to variety of cell surface antigens other than MHC. The MHC antigens are recognized directly by CTL, whereas non-MHC cell surface antigens are recognized by CTL in association with MHC class I gene products on the cell surface (MHC-restriction). Accordingly, there would be several possibilities as to the nature of the target antigens of the F₁ hybrid anti-parental CTL (HyR-CTL). One possibility is that the generation of HyR-CTL occurred in in vitro conditions identical to those in the con-
ventional anti-allogeneic MHC CTL generations. Thus, HyR-CTL would be indeed
directed against an MHC antigen(s) such as H-2K\(^k\) itself. This is, in many ways, the
most attractive hypothesis except that the antigens would not be expressed in the hetero-
zygote. However, recent experimental evidences\(^{31-33}\) suggest that the co-dominant phe-
notypic expression of MHC antigens should be called into question. Furthermore,
Cudkowicz and his colleagues demonstrated\(^{33,21,27}\) that HyR-CTL were capable of
binding to target cells carrying the appropriate H-2 genes in single (heterozygous) or
double (homozygous) dose, and that these HyR-CTL were interestingly capable of lysing
the homozygous but not the heterozygous targets. Therefore, the possibility that the
antigens recognized by the HyR-CTL is MHC class I antigen itself should not be ex-
cluded at this point. It is possible that the HyR-CTL which kill H-2K\(^k\) targets are spe-
cific for but for limited determinants of the H-2K\(^k\) molecule. It is also possible to
speculate that these K\(^k\)-associated determinants on the cell surface are less well displayed
in the F\(_1\) targets, and that their display on the cell surface is in some way strongly in-
fluenced by the cell cycle as corroborated by ourselves.\(^{21}\)

CTL responses to viral and non-MHC minor H antigens have been shown to be
H-2K and/or H-2D restricted.\(^{29,30}\) There are two reasons why I think the HyR-CTL
response that we\(^{18,22}\) have studied is not directed against a minor H transplantation anti-
gen. First, the HyR-CTL response is detectable in a primary \textit{in vitro} culture which
would make it distinct from the anti-minor H response. Second, (B10.BR × B10.D2)F\(_1\)
cells stimulated with AKR/J killed B10.BR as effective as AKR/J targets.\(^{18}\)

Since HyR-CTL are not present in freshly isolated F\(_1\) spleen cells but are seen after
5 days of \textit{in vitro} cultures, the cytotoxicity could possibly be an H-2 restricted activity
directed against fetal calf serum (FCS) antigens absorbed from the culture medium.
However, the presence or absence of FCS in the preparation of target cells and in the
CTL assay system has no effect on the specificity or the level of lysis seen with HyR-
CTL.\(^{19,22}\) Thus, it is clear that FCS components do not serve as the target antigen,
whereas FCS can still be involved in the induction phase of HyR-CTL.\(^{22}\)

We do not yet know the significance of these \textit{in vitro} HyR-CTL in the HyR \textit{in vivo},
but, there is a striking similarity between these \textit{in vivo} and \textit{in vitro} systems as judged
by a number of criteria. These include the fact that \textit{in vivo} and \textit{in vitro} responsiveness
of mice appears at 3 weeks of age,\(^{25-27}\) is attenuated by antimacrophage agents,\(^{24,27}\) is
specifically abrogated by pretreatment of F\(_1\) mice with parental donor cells,\(^{24,26}\) is con-
trolled by non-MHC genes,\(^{18,25-27}\) and so on. There are, however, a number of distinc-
tions between the two responses. The \textit{in vivo} response has a low sensitivity to irradia-
tion\(^{25,27}\) and is thymus independent,\(^{27}\) whereas the \textit{in vitro} counterpart is radiation sen-
titive and the cytotoxic effector is Thy-1-positive T lymphocytes.\(^{18-24,27}\) Recently, our\(^{21}\)
and other\(^{24}\) studies have established that the HyR-CTL response is essentially self-reac-
tive in nature and is inducible spontaneously, under a certain experimental conditions\(^{10-22}\)
in \textit{in vitro} cultures, without addition of specific parental stimulator cells.
3. F₁ RESISTANCE AGAINST PARENTAL LYMPHOCYTE-INDUCED
GVHR-ASSOCIATED IMMUNOSUPPRESSION

It has been established that the GVHR in (A × B)F₁ host injected with parental A or B lymphocytes is initiated by immune responses of donor parental T cells to alloantigens of the opposite parent carried by the F₁ hosts,¹⁴-¹⁵ and that one of the major consequences of the GVHR induced in unirradiated (A × B)F₁ mice is the profound suppression of the humoral⁷⁻⁹ as well as cellular⁷,¹⁰⁻¹⁶ immune responses of the F₁ hosts. As an interesting and practically important phenomenon in lymphoid cell transplants, unirradiated F₁ hosts can resist against GVHR-associated immunosuppression induced by lymphocytes from certain parental strains [F₁ hybrid resistance to GVHR (HyR-GvHR)],¹⁰⁻¹⁶ yet the genetic and cellular mechanisms controlling the resistance are still poorly understood.¹⁰⁻¹⁶ Considering the fact that the immunodeficiency is one of the major consequences of GVHR⁷⁻¹⁶,³⁴ and is thought to be responsible for the development of GvH disease (GvHD),⁷,¹²,¹⁴,³⁴,³⁵ a better understanding of the phenomenon of HyR-GvHR is particularly important for preventing the pleiotropic acute and chronic GVHR syndromes that hamper HLA-matched BM transplantation in man.

GVHR consists of highly complex cellular systems involving multigenic effect. Recently, it was found that in vitro CTL responsiveness of the spleen cells of unirradiated (A × B)F₁ mice was reduced or abrogated by A-induced GVHR but not B-induced GVHR, depending on the parental strain used as lymphocyte donor,¹⁰⁻¹² or, in some strain combinations, depending on the number of parental lymphocytes employed for induction of GVHR.¹⁰,¹² In addition to the GVHR-associated suppression of in vitro CTL responsiveness of F₁ mice, the other consequences of GVHR induced in unirradiated F₁ mice such as the GVHR-associated mortality also varies as a function of the donor versus host combinations studied.¹⁴,³⁶ These experimental observations collectively indicate that non-H-2 gene control on GVHR is involved as elucidated by several models in the other F₁ anti-parental recognition systems.¹⁸,²⁵-²⁷,³⁶,³⁷

As an intriguing effect of H-2D-linked Hh-1 antigen on GVHR, Elkins and Quant³⁸ reported that (B10 × B10.A)F₁ (H-2b/a) hosts resisted the invasion and proliferation of the alloantigen sensitive unit (AASU) of H-2Db homozygous B10.A(2R) donors but not the AASU of H-2Dd homozygous B10.A and H-2Dd/b heterozygous (B10.A × B10.A(2R))F₁ donors. In this particular strain combination, B10.A mice differ from B10.A(2R) mice only at H-2D region of the H-2 complex. Along the same line, Shearer and Pollison¹⁰ indicated that the failure of B10 but not B10.A spleen cells in the induction of suppression of CTL responsiveness of unirradiated (B10 × B10.A)F₁ hosts. Thus the F₁ HyR-GvHR could be due to the natural HyR of the F₁ hosts to H-2D-Hh-1 gene product(s) expressed by H-2Db homozygous parental B10 cells.

Our study¹² with (B10 × A/J)F₁ mice appears to corroborate the results reported by these authors. However, our results¹¹⁻¹³,³⁶ on F₁ hybrids between B10 and partner mouse strains other than A/J¹² or B10.A (unpublished observation) could not be explained by the H-2D linked Hh-1 gene-mediated HyR-GvHR. We¹⁶ have corroborated
the critical role of non-H-2 genes of particular parental strain in the HyR-GvHR and have estimated the number of the non-H-2 loci responsible for it. Thus, F₁ hybrids that had incorporated two or three of the independently segregating non-H-2 loci exerted the resistance against parental lymphocytes expressing a particular H-2 haplotype on the general genetic background of either of the parental strains. In another instance, F₁ hybrids showed the HyR-GvHR against parental lymphocytes of the particular non-H-2 background regardless of their H-2 haplotype. In the latter case, HyR-GvHR is determined most probably by a limited number of recessive genes at the outside of the H-2 complex, and, hence, the HyR-GvHR is due to the capability of the F₁ mice lacking either one of these recessive gene products to recognize and respond to certain antigenic determinants (the gene products) expressed on GvHR-inducing homozygous parental cells. In the former case, a precise mode of the action of non-H-2 genes that determines the HyR-GvHR seems rather tough to be explained and appears to be much complicated. Whatever the mechanisms of non-H-2 gene control on the HyR-GvHR may be, as to the control in the former case, which is functionally associated by genes at outside of H-2 complex and those at the inside of or closely linked to the H-2 complex, I would like to make a proposal that the non-H-2 genes would regulate the expression of the determinants coded for by a gene located close to H-2 as in the cases of diverse phenomena that are in appearance different from, but, in the way of participation of non-H-2 genes, similar to the F₁ resistance to parent → F₁ GvHR or the HyR of irradiated F₁ to parental BM transplants. At present, we neither know about the actual function of the non-H-2 genes, nor have serologic evidence for such hypothetical antigenic determinants coded for by genes linked to the H-2 complex. Indeed, the lack of serologic endorsement for the hypothetical determinants may discount a great deal of our proposal as in the case of hemopoietic histocompatibility (Hh)-gene hypothesis. To consolidate our proposal, exploration of the regulatory function of the non-H-2 genes at the molecular level of the gene products and linkage analysis of the non-H-2 genes will be required in future studies.

As to the cellular mechanisms of GvHR-associated immunosuppression, recent studies indicated that the functionally distinct subsets of alloantigen-activated donor T cells, such as anti-host CTL, anti-host CTL precursors, suppressor T cells, or helper T cells would be the major effector cells in GvHR associated with a variety of pathologic syndromes. At the present, however, the true cellular mechanisms of the suppression are far from being fully understood. For instance, it has been reported that GvHR-induced suppressor cells are either macrophages, suppressor T cells, or as yet unidentified cells. In order to explore the cellular mechanisms of HyR-GvHR, we attempted to dissect the cellular events occurring in the unirradiated F₁ hosts by following cytotoxic activities, including natural killer (NK) cytotoxicity, in the spleens of F₁ hosts undergoing GvHR induced by parental spleen cells.

In GvHR of parental strain → F₁ combinations that induced neither immunosuppression of the F₁ hosts nor generation of the donor-derived anti-host CTL, F₁
host NK cell activity increased only within a very short period after the induction of GvHR, and the activity soon returned to the normal level. These results indicate that the transient but marked excitation of the F₁ host NK cells should overcome the donor-derived allo-aggressive T cells. Quite in contrast, in GvHR of parental strain → F₁ combinations that induced the suppression of a subsequent in vitro CTL responsiveness of the F₁ host spleen cells to third party alloantigens and generation of the donor-derived anti-host CTL as revealed by our improved dye-exclusion microcytotoxicity test, F₁ host NK cells were activated for a longer time, probably to counter the high potential of the donor-derived allo-aggressive T cells. However, the F₁ host NK cells might fail to overcome the proliferation of the donor-derived allo-aggressive T cells because of the genetically determined high potentiality of such donor T cells, and, ultimately, the F₁ host NK cell activity disappeared completely, suggesting defeat of the F₁ host defence. This should result in the immunosuppression of such F₁ hosts and marked generation of the donor-derived anti-host CTL.

NK cell-mediated cytotoxicity is widely accepted as an important first set host cellular defense against neoplastic cells or viral infections; it was also reported that the HyR of irradiated F₁ mice against BM transp'ants was mediated by radioresistant NK cells of the F₁ hosts. In addition, injection of allogeneic immunocompetent cells was reported to cause an increase in the NK cell activity of the recipients. Taking these at face value, the findings described above may be viewed as a sort of lymphocyte warfare between defending host NK cells and alloaggressive T cells in parental donor cells population. These findings, together with those reported previously, argue that the infiltration of functional donor T cells in the host lymphoid tissues is essential for the pathogenesis of the GvHR-associated immunosuppression.

From the fact that the expansion of self-aggressive clones, i.e., parental donor T cells with anti-host cytotoxicity, is the key event for the immunosuppression in parental → F₁ GvHR, a facet of the parental → F₁ GvHR-associated immunosuppression may be viewed as an example of an acute autoimmune reaction, and the clinical symptoms in GvHD with severe immunodeficiency may be regarded as representing an autoimmune disorder.

4. DISCOVERY OF A NEW MINOR HISTOCOMPATIBILITY LOCUS (H-42) BY F₁ CTL

In this section, I will deal with the discovery of a new minor H mouse alloantigen, that was made during the course of the study on the HyR-GvHR, and also with a finding that the mouse CTL response to this newly defined alloantigen is under a novel control of class II MHC gene.

It has been established that GvHR is initiated by immune responses of the donor T cells to alloantigens carried by the hosts and overall outcome of GvHR, such as the HyR-GvHR, is strongly influenced by genes within or very close to the H-2 complex and also by a limited number of genes located outside the H-2 complex. It is also
suggested that both the polymorphic H-2 genes and Igh (immunoglobulin heavy chain) locus would influence the development of the T cell repertoire. In view of these findings, our study was first designed to evaluate contribution of Igh-linked genes to the phenomenon of HyR-GvHR as a candidate of above mentioned non H-2 genes. During the course of the study, we found that the genetic difference at only the Igh locus, which distinguishes C3H.SW/Hz (CSW: H-2^b, Igh^j) mice from the Igh-congenic CWB/13Hz (CWB: H-2^b, Igh^b) mice, can determine the inability of CSW spleen cells to induce the GvHR-associated immunosuppression in (B10.BR × CWB)F_1 (BWF_1: H-2^k/b, Igh^b/b) hosts. It was also demonstrated that spleen cells of BWF_1 hosts under condition of GvHR induced by injection of CSW or C3H/HeN (C3H: H-2^k, Igh^j) spleen cells were primed to generated anti-CSW CTL, and that the subsequent culture of such BWF_1 spleen cells with CSW stimulator cells augmented the CTL activity. The CTL lysed specifically the target cells carrying the Igh^j allele in the context of self H-2K^b molecule, suggesting the CTL activity is directed to an allogeneic minor H antigen coded for by a gene linked to Igh^j. However, determinations of Igh haplotype of the serum IgG and of the susceptibility of the splenic lymphocytes to the BWF_1 anti-CSW CTL on individual backcross mice, which carry either Igh^b/j or Igh^b/b in the context of H-2^b/b or H-2^b/k, showed clearly that the gene, most likely a single gene, coding for the target minor H antigen is not located on chromosome 12, on which the Igh complex lies, but is on chromosome 17, being close to the H-2 complex with distance of approximately 8.5 ± 4.3 crossover units.

Skin or tumor grafts performed in certain combination of mouse strains have indicated the existence of a number of polymorphic minor histocompatibility (H) loci at either side of the H-2 complex. The location of these loci on chromosome 17 and differences in the allele among mouse strains so far reported indicate that perhaps none of these minor H loci are identical to the locus encoding the target minor H antigen for the BWF_1 anti-CSW CTL. Thus, according to the rule of consecutive numbering of minor histocompatibility antigens, we designated the minor H antigen to be recognized by the F_1 anti-CSW as H-42^a, and we tentatively regard the mouse strains that do not express H-42^a antigen on the lymphocytes as possessing H-42^b allele at the H-42 locus.

In any event, the discovery of the difference in H-42 allele between Igh-congenic CSW (Igh^j) and CWB (Igh^b) strains gave us a warning that healthy skepticism is necessary in order to avoid misinterpretation of the data obtained in comparative study on congenic mouse strains, in addition to the warning given by Klein et al.

Finally, I would like to report briefly a study which aimed at elucidating MHC control on the anti-H-42 CTL response. It has been established that the MHC-linked immune response (Ir) gene controls the self MHC-restricted CTL responses. In mice, either H-2-linked class I and/or class II genes participate in self H-2-restricted CTL responses to foreign antigens. However, the mechanisms of such Ir-gene control of the CTL responsiveness remain still elusive. In the anti-H-42^a CTL re-
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Response of the H-42b mice, in vivo immunization of H-42b mice with spleen cells bearing allogeneic H-42a antigen and carrying H-2 complex matched with the H-42b mice failed to prime anti-H-42a CTL but induced stable and specific anti-H-42a CTL unresponsiveness, i.e., tolerance, in the H-42b recipients. In contrast, H-2 heterozygous H-42b recipient mice injected with spleen cells bearing H-42a alloantigen on either of the parental homozygous H-2 haplotype were effectively primed to generate anti-H-42a CTL. The H-2 matched H-42a skin graft to H-42b mice, however, consistently primed anti-H-42a CTL in the H-42b recipients.\textsuperscript{50} The tolerant state of anti-H-42a CTL induced in H-2 compatible H-42b mice by H-42a spleen cells has no precedent example in experimentally induced tolerance to minor H antigens, because of its stability and long-lasting duration. Thus, these results indicate that qualitative difference in the consequences of immunization with a single minor H antigen exists between presentation of the antigen on lymphoid cells and on non-lymphoid tissue grafts, and also that H-2 gene compatibility may participate in inducing tolerance of self H-2-restricted CTL to certain neoantigens, such as viral or tumor-specific antigen, appearing on the surface of autologous lymphoid cells.

5. CONCLUSION

Only a little is known about the genetic as well as cellular control over the natural resistance of heavily irradiated F1 mice against parental BM transplants and of unirradiated normal F1 mice to GvHR induced by parental lymphocytes. The control is apparently complex and polygenic.\textsuperscript{4-6,10-16} Concept of F1 hybrid resistance is becoming more complex,\textsuperscript{18,23,24,57} and the recessive antigen theory\textsuperscript{25,26} now seems far from satisfactory.\textsuperscript{20,51-53,57} I do not believe that the various phenomena in F1 anti-parental reactivity described in the present paper are simply fortuitous and are each other independent. Although, these phenomena still present us with a number of apparent contradictions, they should reflect a final outcome of different cellular events involving multigenic effect. I regard these diverse experimental observations as the different tips of one iceberg. The hidden parts of these tips must somehow be firmly interconnected, and the critical involvement of MHC-linked class I genes, especially H-2D region of the mouse MHC, in many of these independent observations is probably one of these hidden associations. Better understanding of these phenomena will also provide us with ideas to delineate the functional associations of MHC and non-MHC gene products in the immune responses.

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