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Major and minor histocompatibility antigens to NIMA

Prediction of a tolerogenic NIMA effect

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The immunologic effects of developmental exposure to non-inherited maternal antigens (NIMA) are heterogeneous, either tolerogenic or immunogenic. The role of minor histocompatibility antigens (MiHA) in NIMA effects is unknown. We have recently reported that the NIMA effect can be classified into two distinct reactivities, low and high responder, to NIMA in utero and during nursing depending on the degree of maternal microchimerism (MMc) and Foxp3 expression of peripheral blood $CD4^+CD25^+$ cells after graft-versus-host disease (GVHD) induction. These reactivities were predictable before transplantation, using an MLR-ELISPOT (mixed lymphocyte reaction; enzyme-linked immunospot) assay by comparing the number of IFNγ-producing cells stimulated with NIMA. Moreover, this assay was also applicable in both major and minor NIMA-mismatched setting. These observations are clinically relevant and suggest that it is possible to predict the immunological tolerance to NIMA.

Key words: NIMA, tolerance, major histocompatibility antigen, minor histocompatibility antigen, GVHD

Abbreviations: NIMA, non-inherited maternal antigens; MHC, major histocompatibility; MiHA, minor histocompatibility antigens; MMc, maternal microchimerism; GVHD, graft-versus-host disease; MLR, mixed lymphocyte reaction; ELISPOT, enzyme-linked immunospot

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There have been several investigations of NIMA.[1,2] The immunologic effects of developmental exposure to NIMA are heterogeneous.[3-5] The precise mechanisms of the heterogeneity are still under investigation. The relevance of MiHA in NIMA effect has not been reported. Not only in the MHC-identical but also in the MHC-haploidentical situation, MiHA alloreactivities may be induced upon transplantation.[6] Therefore, focusing on the NIMA effect to H-2 is clinically relevant. In addition, it remains to be studied whether or not particular microchimeric cell types are associated with either a sensitized or a tolerized MiHA immunization status.

The mouse MiHA loci confer a wide range of immunogenicity ranging from weakly to strongly immunogenic.[7-9] Recent studies have provided evidence that GVHD could be caused by a limited number of MiHA, including H4, H7, H13, H28, H60 and H-Y.[10-12] This MiHA immunodominance was manifested on genetically varied backgrounds among B10, BALB/c and DBA/2 strain.[7,13,14] So far, there has been no report distinguishing H-2 from MiHA as NIMA. We have classified mouse models of NIMA based on major and minor histocompatibility antigens to NIMA (**Table 1**). In our current study, all B10 congenic mice were used as NIMA model, and those MiHA had matched entirely in this system (**Table 1b**). On the other hand, in the conventional model (**Table 1a**), the NIMA is not only $H\text{-}2^d$ but also noninherited MiHA of DBA/2. Therefore, our NIMA mouse model, but not conventional NIMA model, did not affect immunogenicity of MiHA. We examined the tolerogenic potential of NIMA exposure for H-2 of class I and II disparities without any influences of the MiHA.[15] Contrary to previous reports that showed an apparent NIMA effect,[1,16] we have described no evidence of the NIMA effect. The reason for the difference remains to be determined, but it could be due to the abrogation of the MiHA effect in our system.

Next, we analyzed whether the differences in the effects of NIMA underlie the individual responses to alloantigen by MLR. MLR assay demonstrated that

Table 1. Major and minor histocompatibility antigens to NIMA

Mating combination	Offspring	$NIMA^{H-2}$	$NIMA^{MiHA}$
(a) B6 male x B6D2F1 female (conventional model: major + minor)	$H\text{-}2^{b/b}$	$H\text{-}2^{d}$	Noninherited MiHA of DBA/2
(b) B10.BR male x [B10.D2 x B10]F1 female	$H\text{-}2^{b/k}$	$H\text{-}2^{d}$	none
(major only)	$H\text{-}2^{d/k}$	$H\text{-}2^{b}$	none
(c) C3H male x [BALB/c x B6]F1 female	$H\text{-}2^{b/k}$	$H\text{-}2^{d}$	Noninherited MiHA of B6 and BALB/c
(major + minor)	$H\text{-}2^{d/k}$	$H\text{-}2^{b}$	Noninherited MiHA of B6 and BALB/c

NIMA-exposed mice could be classified into high responders (HR) and low responders (LR). The level of MMc in the LR group was significantly higher than that in the HR group, supporting the recent report in reference 17. Moreover, the NIMA tolerogenic effect was associated with Foxp3 expression and the induction of regulatory T cells in a NIMA mouse model with an H-2-mismatched and MiHA-matched combination. Our results demonstrated that there was an unevenness in the acquisition and maintenance of MMc in offspring in a breeding which excluded any role for differential inheritance of MiHA. This variability amongst offspring was not due solely to differences in MiHA gene inheritance, but seemed intrinsic to mammalian reproduction; i.e., allogeneic pregnancy and nursing itself.[16,17]

Although several reports have described a tolerogenic NIMA effect,[1,16,18] severe GVHD has still been experienced and methods to predict severe GVHD have not been found.[19-21] To further examine the immunological mechanism underlying the tolerogenic NIMA effect in vitro, we have examined the frequency of IFNγ-producing alloreactive cells using an ELISPOT assay combining with MLR (MLR-ELISPOT).[15] We detected a potent response by IFNγ-producing cells from the NIMA-exposed HR group ($H\text{-}2^{d/k}$, $NIMA^{b}$) and NIMA-nonexposed mice ($H\text{-}2^{d/k}$) to B10 stimulator cells as compared with those from NIMA-exposed LR group ($H\text{-}2^{d/k}$, $NIMA^{b}$) (**Table 1b**). Moreover, we described the predictable NIMA tolerogenic reactivity in another NIMA model with major- and minor-mismatched combination (**Table 1c**). These mice were affected by both major and minor NIMA just like the conventional NIMA model.[1,18] Collectively, these results demonstrate that it is possible to predict the immunological tolerance to NIMA in major and minor mismatched transplants.

Finally, NIMA effects directed toward MHC antigen were divided into high (immunogenic) and low (tolerogenic) reactivities. These effects were correlated with MMc and Foxp3 expression. The reactivities were predictable by MLR-ELISPOT assay. Therefore, non-T cell depleted NIMA-mismatched haploidentical transplantation could be performed more safely just by evaluating IFNγ-producing cells of donors against NIMA before transplantation.

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