Review

Theory and Treatment of the X-Inactivation Chimera in Female-Prevalent Autoimmune Disease

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Abstract. The vast majority of patients with systemic autoimmune diseases such as Sjögren’s syndrome and systemic lupus erythematosus are female. The female prevalence of such autoimmune diseases is poorly understood. In theory, X-chromosome inactivation and resultant tissue chimera may explain the female predisposition to systemic autoimmunity. For example, autoreactive T cells may fail to be tolerized by self antigens encoded by one of the two X chromosomes (the Kast conjecture). In the periphery, these autoreactive T cells may stimulate B cells expressing the target X-encoded antigen, so the Kast conjecture can be extended to explain how systemic autoimmunity may be induced. Another hypothesis proposes the X-encoded genes cause autoimmunity by affecting B or T cells directly; however, significant evidence has been raised discrediting this hypothesis. An attractive feature of X-inactivation hypotheses is that the discordance rate between monozygotic twins may readily be explained, because otherwise-identical twins may have different X-inactivation patterns. Reviewed here are the immunobiology of X-inactivation chimera and its roles, both known and postulated, in autoimmune disease. Also discussed are methods appropriate for determining the cellular and molecular targets of autoreactive T cells in female-prevalent autoimmune diseases and methods by which these targets might be used as tolerogens to treat these diseases.

Key words: X-chromosome inactivation; dendritic cells; systemic autoimmune disease; chimeraism; systemic lupus erythematosus; Sjögren’s syndrome.

Chimerism in Autoimmune Disease

It is becoming increasingly clear that many autoimmune diseases may be caused by chimeraism. The most striking examples of chimeraism’s link to autoimmunity are the recent discoveries that long-lived fetal cells may cause systemic sclerosis (scleroderma) and polymorphic eruptions of pregnancy in humans. Other examples of chimeraism’s link to autoimmunity are: 1) bone marrow transplant-induced chimera may initiate graft-versus-host disease; 2) experimentally generated blood chimera can induce antibodies associated with systemic lupus erythematosus; 3) chromosomal chimera resulting from fragile-X syndrome, Bloom syndrome, and heterogenous trisomy have occasionally been reported to be associated with systemic autoimmunity.

Can chimera account for the increased incidence of autoimmunity in women? For chimera to be a general cause of female-prevalent autoimmunity, there must be a mechanism whereby females and not males are chimeric and where neither pregnancy nor engraftment is required. X-chromosome inactivation creates just such chimeraism.

X-chromosome inactivation is the process of condensing one of the two X chromosomes into a hete-
rochromatic “Barr body”. X-inactivation occurs early in development, and the choice to inactivate a particular X chromosome is normally made randomly for each cell of the late blastula. Thus, nearly all mature female tissues are mosaics of two cell types: cells that have inactivated the paternal X chromosome and cells that have inactivated the maternal X chromosome. While normal, XY males do not undergo similar sex-chromosome inactivation, Klinefelter’s males — who have two X chromosomes in addition to the Y chromosome (XXY) — do inactivate one of their X chromosomes and, thus, are equivalent to females in this respect. The theory that X-inactivation chimerism causes the high incidence of autoimmunity in females predicts that Klinefelter’s males should also be associated with a high incidence of systemic autoimmunity (as, in fact, they are).  

X-Inactivation Chimerism in Autoimmune Disease  

Richard Kast was the first to postulate a role for X-inactivation chimerism in female-prevalent autoimmune disease. Kast’s conjecture was based on fundamental principles of self/non-self discrimination. Kast reasoned that, because developing lymphocytes are screened for autoimmunity, if the thymic screening cells exist in two different forms, lymphocytes autoreactive to one but not both of the two cell types may evade tolerance. Kast postulated that X-inactivation created this chimerism of the tolerizing thymic cells.

It was unclear why, given Kast’s conjecture, autoimmunity would not be a general feature of all women (indeed, Kast suggested that X-inactivation is merely a gender-specific risk factor). A different answer was posed once the X-inactivation of the peripheral blood cells was found to deviate significantly from a balanced 50:50 ratio in a subset of control women. With this fact in hand, X-inactivation chimerism could be extended from a simple risk factor to a direct cause of systemic autoimmunity in women. Namely, autoimmune disease would occur when there existed an extreme imbalance in the two “X-types” of tolerizing cells (now known to be the thymic dendritic cells (DCs)). Thus, in women with extremely “X-imbalanced” DC populations, autoreactive T cells could escape negative selection through ignorance of the less frequent DC X-type. One attractive feature of this hypothesis is that it can explain the incomplete penetrance of systemic autoimmune diseases in monozygotic twins because X-inactivation patterns often differ if the twinning event is early.

Of note is the competing hypothesis that autoimmune disease is caused by X-linked genes that exert their effects on T and/or B cells. Based on this hypothesis, X-inactivation differences in these cells are predicted to cause monozygotic twin discordance for disease. This prediction has been tested. When peripheral blood mononuclear cells (PBMCs) were assayed, no significant X-inactivation pattern differences were found between lupus patients and their discordant monozygotic twins or between lupus patients and normal controls. Nor were PBMC X-inactivation pattern differences found to be associated with rheumatoid arthritis or multiple sclerosis. Thus, it appears that the monozygotic twin discordance for these autoimmune diseases cannot be explained by differences in PBMC X-inactivation patterns, and, by extension, the hypothesis that disease is primarily caused by X-linked “autoimmunity” genes that affect B and/or T cells directly may be rejected.

The finding that PBMCs are not X-imbalanced in these diseases has also been cited as evidence that the thymic DCs are likely not to be X-imbalanced either. This assertion is based on the assumption that X-inactivation patterns are equivalent between mature peripheral blood cell types; this assumption has been reported to be incorrect. Thus, neither Kast’s conjecture that the tolerizing cells’ X-inactivation leads to autoimmune disease nor the X-imbalance hypothesis has been tested.

Mouse Models of X-Inactivation-Mediated Autoimmunity  

Unintentionally, the methods employed to generate animal models of autoimmunity may have selected against forms of autoimmunity involving X-inactivation. For example, by establishing inbred animal strains with homogeneous genetic backgrounds (e.g., MRL/lpr/lpr), X-inactivation mosaicism is precluded. On the other hand, in hybrid animals, X-inactivation mosaicism may be involved. Candidates for such animal models are those where hybrid females develop more severe disease than do males, including (SWR × SJL)F1 mice and DBA/2 × (SJL × DBA/2)F1 mice. The best candidate may be the female hybrid (NZB × NZW)F1 mouse, which has an increased frequency of lupus-like symptoms over that of the (NZB × NZW)F1 male and both parental strains. By the X-inactivation mechanism, chimerism of the DC population would allow for X-reactive T cells to survive negative selection. This mechanism can explain a wide range of puzzling observations made in (NZB × NZW)F1 female mice, including the dependence of autoimmunity on helper T cells even while T cell tolerance to self antigens ap-
pears intact. Significantly, none of the self antigens for which T cell tolerance has proven intact are X-encoded. By the Kast conjecture, the “polyreactive” T cells seen in these mice are not polyreactive at all but are instead specifically reactive to an X-chromosome-encoded antigen.

Treatning the X-Inactivation Chimera

Systemic autoimmunity is usually treated with immunosuppression. Recently, autologous stem cell rescue following intensive immuno/myelosuppresion has emerged as a promising experimental treatment. The logic of using autologous stem cells is that the stochastic component of autoimmunity is thought to be more important than genotype, so the reseeding of the bone marrow is believed to re-randomize the stochastic component and “reset the system”. Given that X-inactivation patterns may be that stochastic component, the alteration of the X-inactivation balance may be responsible for the preliminary successes of autologous stem cell replacement. If so, the autologous stem cells used to reseed the bone marrow should be “X-rebalanced” to ensure maximum efficacy.

If X-imbalance of the thymic dendritic cells leads to systemic autoimmunity, systemic autoimmunity may be treated less invasively and more specifically by addressing the X-inactivation defect. For example, DCs may be used as T cell tolerogens. One prediction of the X-imbalance hypothesis is that the DC X-type that primes the autoreactive T cells will be the DC X-type that is less common in the thymus. Thus, if one harvests some of these minority-X-type DCs from a patient and treats these DCs (e.g., with IL-10/9, 25) so that they present self antigens but are unable to prime T cells, these DCs, when restored to the patient, will tolerize the autoreactive T cells. As a practical matter, one may not even necessarily need to separate the dendritic cells on the basis of X-inactivation patterns, because the X-inbalance hypothesis predicts that the majority DC X-type is irrelevant.

More specific would be the identification and use of the self peptides that are recognized by the autoreactive T cells. By the Kast conjecture, these peptides will be expressed differentially depending on the active X chromosome, and by the X-imbalance hypothesis, the target peptides will be those presented exclusively by the minority but not the majority DC X-type. MHC-associated peptides can be isolated from the two DC X-types, or two-dimensional gels can be compared to identify differentially expressed proteins or mRNA transcripts. Once identified, the target peptides may be used as T cell tolerogens.

Alternatively, the peptide targets can be identified using MHC-matched B cells in conjunction with T cells and self antigens from the autoimmune patient. MHC-matched B cells from other patients have been found to be unable to react to these autoreactive T cells, and this inability to react has been attributed to the lack of appropriate self antigens presented by the MHC-matched B cells. Therefore, if these unresponsive B cells are reacted with the autoimmune patient’s self antigens, some of these B cells will be able to bind the self antigens and present derivative epitopes. Thus, the identification of these B cells will be a key step in the identification of the self antigen(s) recognized by the autoreactive T cells. Namely, these B cells may be used to generate monoclonal antibodies that bind and label the actual targets of T cell autoimmunity (the appropriate B cells could be identified simply as those that spontaneously fuse to form hybridomas).

Closing Remarks

Kast’s conjecture that X-inactivation chimerism of the thymic tolerizing cells compromises negative selection remains promising but untested, and the evidence supporting the conjecture is circumstantial but strong. Extreme X-imbalance in the thymic DC population has been predicted to cause autoimmunity, and the X-imbalance hypothesis predicts that differences in the X-inactivation patterns between monozygotic twins will account for disease discordance. The basic components of the Kast conjecture cannot hold for inbred mouse models of autoimmunity, because homogeneous genetic backgrounds disallow meaningful X-inactivation chimerism, but hybrid mouse models such as (NZB x NZW)F1 are plausible candidates for X-inactivation-associated autoimmunity. The DC X-inactivation patterns may be exploited to isolate the direct cellular and molecular targets of autoimmunity in patients. These targets can be used, in turn, as T cell tolerogens to treat disease. In conclusion, X-inactivation chimerism may account for the induction of systemic autoimmunity, its discordance between twins, and its female prevalence. Treatments targeting X-inactivation chimerism, though untested, are technically attractive because they rely on novel applications of existing techniques rather than on novel techniques.

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