Manuscript ID IMLET-D-21-00256 Revised Version

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Pitfalls in establishing mouse model of female infertility by immunization with human centromere protein

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Acknowledgements

None.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Authors' contributions

YM, YY and HK were all major contributions in crafting the commentary. TT and MA were major contributors in editing the commentary. All the authors read and approved the final manuscript.

Competing interests

The authors report no conflicts of interest.

Keywords: anti-centromere antibody, centromere protein, epitope, immunization, infertility, internalization

Autoantibodies against intracellular antigens are commonly found in a number of systemic autoimmune rheumatic diseases (SARDs), although the direct pathogenic roles of autoantibodies are still debated [1, 2]. The association between autoantibodies and reproductive failure has been discussed recently [3, 4]. The anti-centromere antibody (ACA), which is an anti-nuclear antibody (ANA), has been argued to be associated with infertility [5, 6]. We present two major pitfalls in experimentally establishing a mouse model of female infertility that harbors ACA.

ACA reacts to three major target autoantigens; centromere proteins (CENPs)-A (Fig. 1A), -B and -C, which always exist in the centromere region during cell cycles [7, 8]. In rheumatology clinics, ACA is often found in sera from patients with systemic sclerosis [9]. For patients suspected of having SARDs, ANA screening is often performed by indirect immunofluorescence (IIF) studies using the human HEp-2 cell as a substrate. ACA can be demonstrated by the characteristic staining pattern of discrete speckles scattered in interphase cells and aligned at the chromatin mass on mitotic cells (Fig. 1B) [10]. Very recent study has shown that mice in which ACA is produced by immunization with a commercially available human CENP-A protein in an adjuvant produce ANA that shows nucleoplasmic speckled staining without the chromosomal staining of dividing cells [11]. Centromere antigens are highly conserved among species. For example, amino

acid (a.a.) sequences of CENP-B have 92.1% identity between humans and mice [12]. For CENP-A, however, the 38 amino-terminal a.a. sequences have only 60.0% identity between humans and mice (Fig.1A). Crucially, our previous study and others [13, 14] demonstrated that epitopes of ACA in CENP-A exist only at the amino-terminal portion of CENP-A. It seems highly unlikely that mice immunized with human CENP-A protein would produce ACA that reacts to mouse CENP-A. Several studies have successfully established mouse models of human SARDs by immunization with human autoantigens [15-23]. In these studies, the a.a. sequence identities between humans and mice for the autoantigens SRP, HMGCR and topoisomerase I are 99%, 100% and 97%, respectively. The first major pitfall in experimentally establishing the mouse model in question is the differences in CENP a.a. sequences between humans and mice.

Furthermore, Fan *et al.* reported that the immunization of mice with CENP-C caused aberrant chromosome segregation during oocyte meiosis [24]. They used commercially available human recombinant CENP-C and showed mouse antibody to exist in oocytes from CENP-C-injected mice. Numerous studies have shown that different autoantibodies can enter living cells, ever since the study by Alarcón-Segovia *et al.* [25-38]. Fan *et al.* used a commercially available ELISA kit (Euroimmun, Lübeck, Germany) to detect ACA in sera from mice [24]. It should be noted that this ELISA kit uses

recombinant human CENP-B protein, and not CENP-C. Fan’s group note that the CENP-C antibody was found by ELISA only in serum from the experimental mice, but this is misleading. Autoantibodies in model mice should be carefully characterized in detail.

We would like to address the second important pitfall concerning experimental autoimmune mouse models carrying ACA. There are significant structural differences in the centromere between humans and mice. Mouse chromosomes, with the exception of the Y chromosome, are telocentric [39], whereas human chromosomes are meta/submeta/acrocentric [40]. The human centromere contains tandem repeats of 171-bp α-satellite monomers organized head to tail into higher-order repeats (HOR), whereas the mouse centromere is made up of major satellite sequences of 234-bp monomers (spanning ~6 Mb) and minor satellite sequences of 120-bp monomers (spanning ~600 kb) [41]. Moreover, each human chromosome has a different number of monomers that make up its HOR, with some chromosome-specific sequences contained within the homogenized array [41]. These differences in centromere structure between the two species make it difficult to interpret the mechanism whereby mouse models mimic human diseases. Two previous studies using human or mouse cells independently showed that the injection of human ACA had similar effects of interference with chromosome congression for human and mouse cells, but demonstrated different results between

humans and mice for nocodazole-arrested cells [42, 43]. When experimentally establishing an animal model of female infertility that harbors ACA, it might be necessary to use non-murine species.

Although we are very interested in future work on experimental autoimmune mice immunized with mouse CENP-A and the impairment of their oocytes, the induction of experimental autoimmune diseases by immunization with an autoantigen is limited due to self-tolerance [45, 46]. In breaking immune tolerance, the immunization of animals with the deleted protein knocked out is a powerful approach [47]. Unfortunately, CENP-A knockout is lethal in mice [48]. Chemically modified partial CENP-B may be a good candidate immunogen for breaking self-tolerance, because the immunization of fusion protein of C-terminal partial CENP-B with βgal establishes monoclonal ACA [49]. Infertility animal models with ACA promise to be useful for investigations into pathogenic epitopes for ACA.

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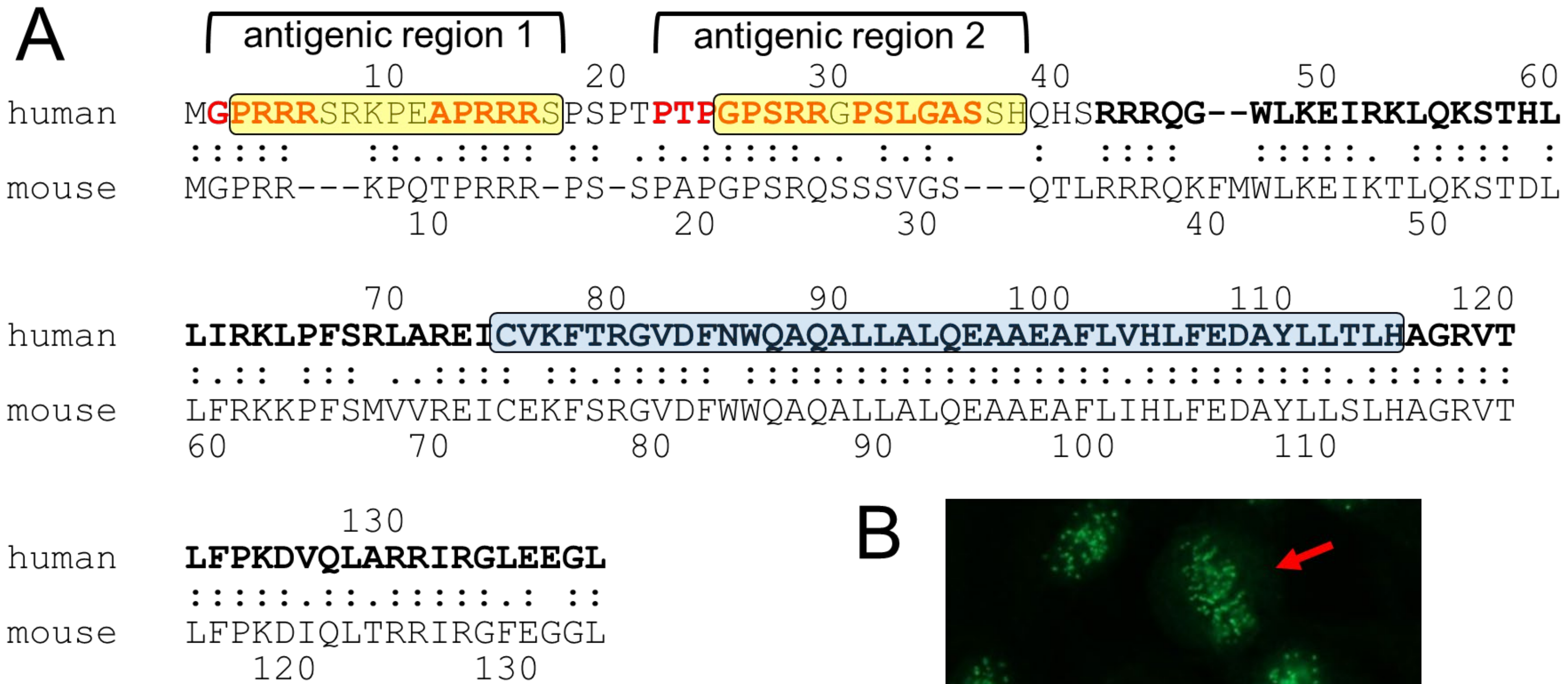
Figure legend

Fig. 1 Amino acid sequences of human and mouse CENP-A and immunohistochemistry of anti-centromere antibodies (ACA). (A) Alignment of human and mouse CENP-A amino acids. Yellow boxes show the two CENP-A autoepitopes demonstrated by our previous study using synthetic peptides [13]. Amino acids in red font constitute amino acid core sequences of autoepitopes, and the antigenic regions 1 and 2 were proposed by Mahler et al. [14]. Characters in bold black font indicate the histone H3-like domain, which is not reactive to ACA. The blue box shows the CENP-A targeting domain [49]. (B) Human ACA stains as discrete speckles scattered in interphase cells and aligned at the chromatin mass on mitotic cells (arrow).

Figure 1



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