Collagen type VI is the antigen recognized by the ER-TR7 antibody

Since its first description published in this journal in 1984 [1], the monoclonal antibody ER-TR7 has been used in a great number of immunological and non-immunological studies. Even so, its antigen is still unknown. The ER-TR7 (Erasmus University Rotterdam-Thymic Reticulum) antibody was originally produced in a screen aimed at identifying monoclonal antibodies reactive against non-lymphoid stromal cells of the mouse thymus [1]. Therefore, its staining pattern in secondary lymphoid organs was characterized in particular detail. Staining of mouse spleen with ER-TR7 revealed a system of extracellular reticular fibrils that delineates the microanatomical architecture of the organ and allows a clear distinction of the red and white pulp and their interconnecting marginal zone. Other structures that contain extracellular matrix (ECM) components, such as the capsule, trabeculae, central arteriole, and other vascular walls were also strongly labeled by ER-TR7. Similarly, a fine reticular ER-TR7 staining delineated the T and B cell microenvironments within lymph nodes [2]. In the same lymphoid organs, ER-TR7 also marked the stromal cells known as Fibroblastic Reticular Cells (FRCs), which are responsible for the secretion of the ECM proteins forming the reticular fibers. Further studies showed that FRCs dynamically regulate the molecular composition of the reticular fibers that are in turn essential for a proper immune response [3]. Notably, injection of the ER-TR7 antibody in mice resulted in microanatomical alterations of lymph nodes and disturbed lymphocyte migration, leading to defects in tolerance development in an allograft mouse model [4]. Another particular function of the reticular fiber system is the delivery of small molecules such as cytokines, antigens, and Igs to specific compartments of secondary lymphoid organs. Indeed, reticular fibers have a specialized “conduits” architecture characterized by a cell-free lumen that is filled by three distinct layers of ECM proteins: an outer basement membrane, a collagenous core and a microfibrillar layer in between. Conduits are, in turn, ensheathed by FRCs. Interestingly, ER-TR7 staining is invariably found, together with stainings for fibrillin-1 and 2, and in the microfibrillar layer of these conduits in different compartments of both lymph nodes and spleen [5].

Several subsequent studies have reported that ER-TR7 not only labels the stroma of secondary and primary lymphoid organs, but that the same unknown antigen is ubiquitously distributed in the extracellular space of connective tissues, therefore supporting its use as a fibroblast marker in virtually every tissue of the organism. For example, ER-TR7 positivity was found to be abundant in the blastema of the regenerating digit tip [6], massively upregulated by adventitial fibroblasts in the fibrotic thoracic aorta of mice injected with angiotensin-II [7], or used to distinguish between fibrogenic and myogenic fate in cells derived from mouse skeletal muscle [8]. However, despite this widespread utilization, the antigen recognized by ER-TR7 remains unknown and Van Vliet et al. originally reported that ER-TR7 did not react with purified laminin, fibronectin, collagens I-V or nidogen [2].

While studying the molecular composition of spleen reticular fibers, we noticed that the staining pattern of ER-TR7 greatly overlaps with that of collagen VI. Collagen VI is a ubiquitous ECM protein forming a network of beaded microfibrils mainly assembled of α1, α2, and α3 chains. Therefore, we hypothesized that collagen VI is the unknown antigen recognized by ER-TR7. To test our hypothesis we co-stained spleen and mesenteric lymph node sections of WT and collagen VI α1-KO mice [9] using ER-TR7 together with antibodies specific for the α1 and α3 chains of collagen VI and another microfibril-associated protein, EMILIN1 [10]. In WT samples, all antibodies revealed the typical reticular fiber network, with a largely overlapping pattern. As expected, no extracellular staining was detected with the collagen VI specific antibodies in the knockout sections. Importantly, the ER-TR7 signal was also completely abolished, while EMILIN1 was still detected in the reticular fibers of collagen VI α1-KO spleen and lymph node sections (Fig. 1A and 1B). Western blot of spleen extracts with a collagen VI α1 antibody confirmed the lack of the α1 chain in KO tissues, but no signal could be detected under reducing or non-reducing conditions with the ER-TR7 antibody on either WT or KO samples, indicating that ER-TR7 does not work in this method, probably due to the epitope being conformation-dependent (not shown). To
confirm these results in an independent system, we generated collagen VI α2-KO cells by targeting the third exon of the COL6α2 gene in the murine MC3T3-E1 cell line via CRISPR/Cas gene editing (gRNA sequence: GGGGTTGGTCAACTTCGCG). Western blot analysis of cell extracts confirmed the lack of the α2 chain and an acrylamide-agarose composite gel system was used to further confirm the absence of secreted high-molecular weight collagen VI tetramers in the conditioned medium of α2-KO cells (Fig. 2A). Similarly to what was observed with the tissue extracts, we did not detect any specific band with the ER-TR7 antibody in the cell layer or conditioned medium of control MC3T3-E1 cells (not shown). However, immunofluorescence staining of WT cells showed extracellular ER-TR7 staining of ECM fibrils that overlapped completely with the collagen VI fibrillar network. Finally, the same fibrils were not detected in the ECM of α2-KO cells by ER-TR7 and collagen VI α1 and α3 specific antibodies, while the EMILIN1 staining was comparable in control and KO cells (Fig. 2B).

Altogether, these observations show that native collagen VI is the conformation-dependent molecular antigen recognized by the ER-TR7 antibody.

In conclusion, collagen VI is the bona fide antigen recognized by ER-TR7 and data generated with the use of this antibody can be reassessed in view of our new discovery. Moreover, these findings suggest that further investigation of the role of collagen VI in lymphoid organ structure and function would be relevant.

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Figure 2. Analysis of WT and collagen VI deficient osteoblasts using the ER-TR7 antibody. (A) Cell lysates of WT and collagen VI α2-KO cells were probed with an α2-specific antibody in western blot under reducing conditions. Conditioned media from the same cells were also analyzed by electrophoresis on 0.5% agarose/2.4% polyacrylamide composite gels under nonreducing conditions and immunoblotted with an antibody specific for the α3 chain of collagen VI to detect secreted collagen VI tetramers (molecular weight of approximately 2000 kDa). Thyroglobulin (660 kDa) was used as a molecular weight reference. Data representative of 2 experiments. The original uncropped blots are provided in the Supporting Information. (B) Confocal immunofluorescence microscopy for ER-TR7 (magenta), collagen VI (green), and EMILIN1 (red) in the ECM produced by MC3T3-E1 murine osteoblasts. Note that lack of the α2 chain leads to intracellular retention of the α1 chain. MC3T3-E1 cells were plated at a density of 4 × 10⁴ cells/cm² on glass coverslips and grown for 5 days in the presence of ascorbate. Two independent experiments using three different α2-KO cell clones were performed with similar results. Scale bars: 100 μm.

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References
1 van Vliet, E. et al., Eur. J. Immunol. 1984. 14: 524–529.
2 Van Vliet, E. et al., J. Histochem. Cytochem. 1986. 34: 883–890.
3 Krishnamurty, A. T. and Turley, S. J. Nat. Immunol. 2020. 21: 369–380.
4 Burrell, B. E. et al., Transplantation. 2015. 99: 1119–1125.
5 Lokmic, Z. et al., Semin. Immunol. 2008. 20: 4–13.
6 Marrero, L. et al., Regeneration. 2017. 4: 69–84.
7 Rateri, D. L. et al., Am. J. Pathol. 2014. 184: 2586–2595.
8 Brack, A. S. et al., Science. 2007. 317: 807–810.
9 Bonaldo, P. et al., Hum. Mol. Genet. 1998. 7: 2135–2140.
10 Schiavinato, A. et al., J. Invest. Dermatol. 2016. 136: 1150–1160.

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Abbreviations: ECM: extracellular matrix • FRC: fibroblastic reticular cell

Additional supporting information may be found online in the Supporting Information section at the end of the article.