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Letter to the Editor

Mapping and sequencing of the murine 'tissue' Transglutaminase (*Tgm2*) gene: absence of mutations in MRL*lpr/lpr* mice

Dear Editor,

The transglutaminase family includes both intracellular and extracellular enzymes catalyzing Ca^{2+}-dependent reactions which result in the post-translational modification of proteins at the level of glutamine and lysine.[1] This post-translational modification leads to the formation of the $\varepsilon(\gamma$-glutamyl)lysine cross-links and/or to the covalent incorporation of polyamines into proteins.[2] The formation of these covalent cross-links leads to the polymerization of substrate points which become very resistant to physical-chemical stress.[1]

The 'tissue' transglutaminase (E.C. 2.3.2.13) or type-II transglutaminase gene (*Tmg2*) codes for a protein which, in mammals, has a molecular weight of about 80 kDa. Tissue transglutaminase is constitutively expressed in a few cell types localized in specific tissues[3] and its enzymatic activity seems to be essential for a correct program of apoptosis.[4,5] Transglutaminase-catalysed protein cross-linking enhances the stability of apoptotic bodies, reducing their leakage. This may prevent inflammatory responses as well as provide dissequestration of self antigens which could lead to the development of autoimmunity.[6] Notably, a major role for 'tissue' transglutaminase has been proposed in the insurgence of autoimmune Celiac Disease as well as in some neurodegenerative disorders characterized by polyglutamine expansions.[7,8] Recently, Piredda *et al* reported that an inactive form of tissue transglutaminase accumulates in lymphoid organs of MRL *lpr/lpr* mice, which are characterized by a lymphoproliferative autoimmune disorder strongly resembling human Systemic Lupus Erythematosus (SLE).[6,9] These mice carry a mutated inactive form of the CD95-Fas antigen, a protein that plays a key role in apoptosis; nonetheless, this defect can only partially explain the pathogenesis of the *lpr* phenotype and other components must play a role in conferring genetic susceptibility.[9] We tested whether polymorphisms or mutations in the tissue transglutaminase (*Tgm2*) gene account for the observed accumulation and/or the reduced activity of this enzyme in lymphoid organs of *lpr* mice. Total RNA was extracted from thymus and lymphnodes of MRL*lpr/lpr* mice and retrotranscribed with Superscript II (Gibco BRL), using a *Tgm2*-specific primer mapping in the 3′-UTR region of the gene (TGase3 5′-ACCTCTAGATTGAGTCTGGGAAGGGGTCC-3′). Full-length Tgm2 cDNA was obtained by PCR, amplifying 2 μl of the RT reaction with rTth DNA polymerase (Perkin Elmer), basically according to manufacturer's protocols, by using TGase3 and a 5′-UTR mapping primer (TGase5 5′-ACCAAGCTTCTGAGCTGTCGCCGC-TAGC-3′). The PCR product was then cloned into pUC21 vector and sequenced with Sequenase (Amersham). Surprisingly, after comparison with the GenBank database entry for murine tissue transglutaminase (account M55154), we found many discrepancies with the reported *Tgm2* sequence;[10] there were 35 single nucleotide differences, corresponding to 15 amino acid changes, and three insertions which introduced an additional residue and caused a four-amino acid change in the encoded protein. This unexpectedly high number of differences prompted us to re-sequence Tgm2 cDNA from three control mouse strains, namely CBA, C57BL/6 and Balb/c, and from the MRL+/+ background strain from which the MRL*lpr/lpr* were derived. After cDNA cloning by the described procedure, Tgm2 sequencing revealed no differences within the entire coding region both among these strains and when compared to MRL*lpr/lpr* mice. Accordingly, inconsistencies with the

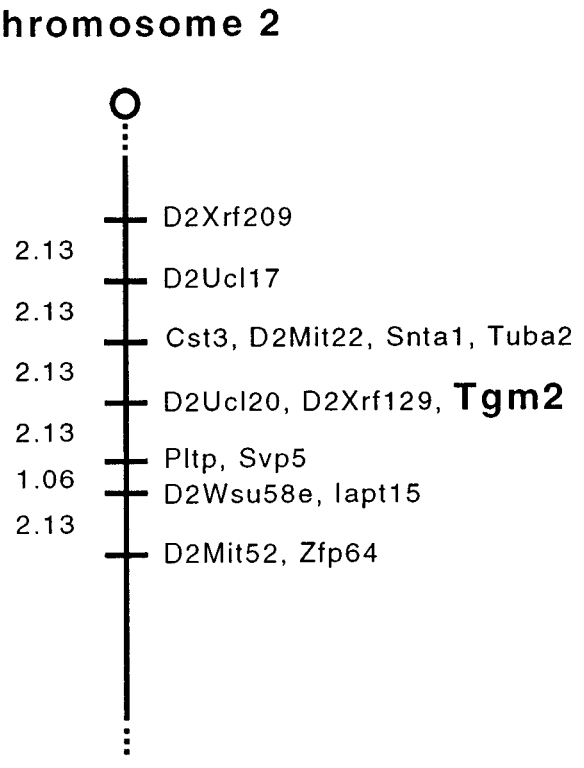

Figure 1 Genetic map of *Tgm2* gene on the distal end of mouse Chromosome 2 (centromere at the top). Numbers on the left show recombination values ± S.E. (cM). Two missing typings were inferred from surrounding data where assignment was unambiguous. Raw data from The Jackson Laboratory were obtained from the World Wide Web address http://www.jax.org/resources/documents/cmdata

GenBank M55154 sequence should be attributable to previous sequencing errors.[10]

Comparison of the newly described mouse Tgm2 sequence with human and bovine tissue transglutaminase shows a higher degree of homology (91.1 and 90.4 *vs* 90.6%, 90% for M55154 sequence). However, this observation rules out defects in the *Tgm2* gene as cause for findings of Piredda *et al*[6] and seems to exclude tTG as a candidate for genetic susceptibility to the MRL*lpr/lpr* phenotype.

As a further characterization of this locus, we then proceeded to *Tgm2* genetic mapping by using The Jackson Laboratory BSS interspecific backcross panel [(C57BL/6JEi × SPRET/Ei)F_1 × SPRET/Ei], consisting of one C57BL/6JEi, one SPRET/Ei and 94 N_2 individuals' genomic DNA. DNAs from C57BL/6JEi and SPRET/Ei were amplified with primers tTG1 (5′AGGCCAGACCTACAGCCG-3′, sense) and tTG2 (5′-GCATGACTTTGGGGCAAGTCAG-3′, antisense), yielding a DNA fragment containing part of both intron 8 and exon 8 of the mouse tissue transglutaminase gene (V De Laurenzi, Biochem. Lab., IDI-IRCCS, Dept. Exp. Med. Univ. Rome Tor Vergata, personal communication). The 1144 bp PCR products were directly sequenced with PCR Cycle Sequencing kit (Perkin Elmer) and, among others, two nucleotide differences were found between the two strains that were used to type the entire panel by PCR and oligonucleotide hybridizations. These differences were two transitions (an A to G and a C to T) located 228 and 235 basepairs into intron 8, respectively. The genotyping experiment assigned the Tgm2 locus to the distal region of mouse chromosome 2 (Figure 1), which is syntenic to human chromosome 20q12 where its TGM2 homolog maps.

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