CD247 variants and single-nucleotide polymorphisms observed in systemic lupus erythematosus patients

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Abstract

SLE is associated with a deficiency in cluster of differentiation 247 (CD247, also known as CD3 zeta chain), a component of the T-cell receptor (TCR)-CD3 complex. A comprehensive analysis showed that in more than half of SLE patients tested CD247 expression was either attenuated or absent. Recent evidence suggests that these variations in expression profiles may be due, at least in part, to polymorphisms in the CD247 gene. Aberrant CD247 transcript variants displaying either spliced exon 7 or short 3’-untranslated region have been detected in SLE T cells, and a recent genome-wide association study reported the existence of new CD247 single-nucleotide polymorphisms in SLE patients. Here, we review these unique and significant features of defective CD247 observed in SLE.

Key words: systemic lupus erythematosus, T-cell receptor, signal transduction, CD247, splice variants.

Introduction

SLE is a prototype autoimmune disease characterized by an abundant production of autoantibodies and the subsequent formation of immune complexes that lead to tissue damage and clinical phenotypes such as butterfly rash and GN [1–3]. The factors of this pathogenic process are thought to be multiple and complex. For example, plasmacytoid dendritic cells activated by the innate immune system produce high levels of type I IFNs (IFN-α and IFN-β) in SLE patients. Type I IFNs affect myeloid dendritic cells and produce a number of other pro-inflammatory cytokines, resulting in the activation of immune cells such as T cells [2, 4].

T cells play a central role in both acquired immune system and immune tolerance and have been shown to be involved in various abnormalities and dysfunctions in SLE patients [4]. Functional activation of T cells is dependent on their surface expression of unique T-cell antigen receptor–cluster of differentiation 3 (TCR-CD3) complexes. TCR-CD3 complexes consist of the alpha and beta chains of TCR, associated with two epsilon, one gamma and one delta chains of CD3 and with a zeta chain [also known as CD3-zeta, TCR zeta chain or cluster of differentiation 247 (CD247)]. Here, we focus on CD247 abnormalities in SLE patients, with particular attention to gene variants and single-nucleotide polymorphisms (SNPs), and discuss how these abnormalities develop into SLE from an immunopathological perspective.

Defective CD247 expression in SLE T cells

CD247 plays an important role in coupling antigen recognition to several intracellular signal transduction pathways. Our early immunoblotting analysis showed that 54.5% of SLE patients (24 out of 44) had lower (>2 s.d.) levels of CD247 protein than did healthy controls. CD247 expression, which seems to be disease-specific in the disease controls (including RA, SSc and primary SS), was not decreased. Among 44 SLE patients, CD247 expression decreased stably in 21 cases and transiently in the remaining three, suggesting the existence of several mechanisms leading to CD247 defect (Fig. 1). The relationship of CD247 expression and SLEDAI with the amount of corticosteroid administered was not significant. Furthermore, direct comparison between active and inactive phases in SLE patients showed no change in CD247 expression [5]. A decrease in TCR-initiated tyrosine phosphorylation was observed in peripheral blood T cells of SLE patients. CD247 protein expression in T-cell subpopulations, including CD4+, CD8+, CD45RA+ (naïve phenotype) and CD45RO+ (memory phenotype), was decreased. The mean CD247 fluorescence intensity in all subpopulations demonstrated a remarkably similar decrease. These results confirm the defective expression and altered tyrosine phosphorylation of CD247 in a large...
proportion of SLE patients, suggesting that defective expression may play an important role in SLE T-cell dysfunction [5].

In normal T cells, the TCR–CD3 complex induces intracellular signalling cascades that lead to normal T-cell function (Fig. 2), while in SLE patients diminished CD247 protein expression [6, 7] undermines the TCR–CD3 complex signalling, leading to T-cell dysfunction such as hyporesponsiveness and decreased IL-2 production, resulting in an overall immune tolerance failure.

Fig. 2 Defective expression of CD247 in SLE T cells.

In normal T cells, signals through the TCR–CD3 complex are transduced into internal cascades, resulting in normal T-cell function while defective expression of CD247 is observed in SLE T cells. Consequently, aberrant signalling causes T-cell dysfunction such as hyporesponsiveness and a decrease in IL-2 production, which leads to immune tolerance failure.

CD247 splice variants in SLE T cells

RNA splicing is the process by which pre-mRNA is converted into mature mRNA by removal of introns and joining of exons. Variations in splicing of the same pre-mRNA can result in the generation of splice variants that display different exon combinations.

Human CD247 is located in chromosome 1 (1q22–q23) and consists of eight exons (Fig. 3). The existence of abnormal CD247 transcripts was previously reported, including splice variants lacking exon 7 and variants with a short 3'-untranslated region (UTR) [5, 7, 14, 15], both of which were exclusively observed in SLE patients [16]. Other variants such as eta (exons 1–7 plus exon 9, see Fig. 3) and iota (exons 1–7 plus exon 10, not shown) are generated by alternative splicing of CD247.

The role of unique splice variants in defective CD247 expression

In vitro analysis of CD247 in SLE T cells showed that mRNA instability was responsible for the lower protein expression of both the short 3'-UTR and the exon 7(-) variants. Furthermore, a T-cell transfectant model with these variants showed similar functional defects to those seen in SLE T cells [8, 15, 17–19].

Mice bearing reduced immunoreceptor tyrosine-based activation motif (ITAM) domains in CD247 similar to those of mutated CD247 produced a substantial amount of cytokines including IFN-γ [20], which suggests that CD247 defects are linked to IFN-γ signature expression. IL-2 production from splenic T cells with all these six ITAMs of CD247 mutated was reduced in the same murine model. This is similar to human SLE T cells stimulated in vitro.

Although CD247 expression levels in SLE patients were found to be inversely correlated with levels of IFN-γ, both in serum and in vitro [21], microarray analysis of mouse transfectants carrying the human spliced variant did not detect any IFN-γ signature [22]. Further investigation on the clinical and experimental aspects of SLE will therefore be needed.

CD247 single SNPs and genome-wide association studies

The mechanism responsible for the generation of spliced CD247 variants in SLE patients is not yet fully understood, and conflicting observations have been reported regarding the presence or absence of mutations or deletions in the 5'-flanking region of the CD247 gene [15, 23]. Splicing donor and acceptor sites have been reported to carry no such polymorphisms [24]. The National Center for Biotechnology Information database currently harbours

Fig. 1 Defects of CD247 expression in SLE patients.

Percentage of decrease in CD247 protein less than mean ± 2 s.d. of healthy controls is shown. In 21 patients, it was stably decreased and in three patients it was transiently decreased. In total, defective CD247 expression in 54.5% of SLE patients were observed [4]. Adapted from Autoimmunity 2005;38:339–46.
seven CD247 gene SNPs that are known to be associated with systemic autoimmune diseases (http://www.ncbi.nlm.nih.gov/gene/919).

Two groups reported the existence of SNPs in the CD247 3'-UTR region [25, 26] (Fig. 4). They showed that the minor alleles of two of these SNPs were causal variants associated with low CD247 expression and that one-third of their mRNA was identical to that of the major alleles. The haplotype carrying the low-expression variants predisposes carriers to develop SLE [25].

CD247 was recently shown to be associated with SLE in Asian populations. A genome-wide association study in people of Chinese ethnicity identified two SNPs (rs858543 and rs704853) in the 78-kb intron 1–2 region, one of which
(rs704853) was linked to oral ulcers, haematological disorders and anti-dsDNA antibody production [27].

Two meta-analyses on RA [28, 29] and a study on systemic sclerosis [30] have reported two CD247 SNPs located in the intron 1–2 region, one associated with RA and the other with SSc. Future analyses should focus on the functional influences of these SNPs on CD247 expression. The strength of effect of known polymorphism may not be substantial, and therefore, variation in CD247 expression must act in concert with other defects.

Conclusions

CD247 splice variants are associated in SLE with aberrant expression through either ITAM deficiency such as exon 7(−) or mRNA instability. Although the molecular mechanisms of RNA splicing are not yet fully understood, various RNA processing dysfunctions, including splicing abnormalities, were recently identified in neurological diseases [31]. We discussed here that abnormal RNA splicing processes were also found to be important in SLE pathogenesis, which suggests that more attention should be focused on new RNA-dependent diseases. Genome-wide analysis of splice variants using high-throughput sequencing and RNA processing functional assessments may improve current understanding of the topic.

Rheumatology key messages

- In SLE, defective expression of CD247 leads to T-cell dysfunction.
- CD247 splice variants and SNPs may play a key role in SLE pathogenesis.

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References

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