Contribution of impaired DNASE1L3 activity to anti-DNA autoantibody production in systemic lupus erythematosus

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Abstract

Anti-DNA autoantibodies are pathogenic in systemic lupus erythematosus (SLE). Cell-free chromatin associated long DNA fragments are antigens for anti-DNA antibodies. In health state, released by cell death and actively secreted by live cells, these cell-free DNA are cleared by deoxyribonucleases (DNASEs). In SLE, cell-free DNA are accumulated. The defective clearance of long fragments of cell-free DNA in SLE is largely attributed to impaired deoxyribonuclease 1 like 3 (DNASE1L3). DNASE1L3 null mutation results in monogenic SLE. The SLE risk single-nucleotide polymorphism (rs35677470) encodes R260C variant DNASE1L3, which is defective in secretion, leading to reduced levels of DNA-SE1L3. In addition, neutralizing autoantibodies to DNASE1L3 are produced in SLE to inhibit its enzymatic activity.

Keywords

systemic lupus erythematosus • DNA • deoxyribonucleases

Introduction

Systemic lupus erythematosus (SLE) is a chronic multisystemic autoimmune disease serologically characterized by various pathogenic autoantibodies. Anti-double stranded deoxyribonucleic acid (dsDNA) antibodies specifically have long been implicated in the pathogenesis of SLE. Described as DNA-reacting factor in 1957, Ceppellini et al.[1] isolated serum from a patient with acute SLE that reacted to DNA extracts from different human and non-human samples. It was unclear at that time whether the components reacting with DNA were antibodies or materials of nuclear origin forming a complex with DNA. Subsequent studies identified these components as circulating anti-DNA antibodies. Tan et al.[2] further demonstrated the presence of not only anti-DNA antibodies but also cell-free DNA in the circulation of SLE patients. Presence of cell-free DNA in the sera of SLE patients was first shown by immunodiffusion assay (Figure 1) and was confirmed by chemical methods. Interestingly, the cell-free DNA were largely un-degraded. Clearly, cell-free DNA in the circulation of SLE patients can serve as antigen for detecting DNA antibodies in vitro and further raised the notion that these cell-free DNA also react with anti-DNA antibodies in vivo. This underscored the foundation to the now well-known pathogenic dsDNA-anti-DNA complexes seen in SLE.

While it is still debatable as to how the production of anti-DNA antibodies is ignited in SLE patients and what ignites it,[3] there is growing evidence that failure in clearance of cell-free DNA by deoxyribonucleases (DNASES), in particular deoxyribonuclease 1 like 3 (DNASE1L3), can lead to production of anti-DNA antibodies and SLE.

Cell-Free DNA as Autoantigen for Anti-DNA Antibody Production

It is unclear whether DNA have evolutionarily had a mechanistic advantage in providing a favorable immune response. It may seem that way as prior studies have shown the relative ease with which B cells can interact with DNA and potentially generate these autoantibodies.[4] Simple structural features such as positively charged residues in variable chains of immunoglobulins can sometimes be sufficient to bind to DNA and thereby form an active immune complex.[5] When cell death occurs due to various pathological conditions including apoptosis or necrosis in malignancy and infection from physiological conditions such as pregnancy, circulating cell-free...
DNA can often be detected. They transiently induce anti-DNA antibodies though they do typically occur at low titers and have not been shown to promote clinical autoimmunity.

Anti-DNA responses can also occur with nucleic acids from infectious pathogens. Bacterial biofilm, which contains complexes of DNA and protein, have triggered autoantibody production in animal models. However, much of our circulating cell-free DNA is thought to be self-derived. Certain cellular processes also enhance extravasation of intracellular DNA. In addition to the various forms of cell death, formation of neutrophil extracellular traps (NETosis) – a release of neutrophilic cytoplasmic complex of nucleic acids and chromatin into extracellular space in an effort to physically contain potential pathogens or detrimental materials – exposes large amounts of protein bound DNA into circulation (Figure 2). Mitochondrial DNA incorporated in NETosis have especially been implicated as targets for autoantibodies. NETosis can thus directly facilitate the availability of nucleosomes for the formation of anti-dsDNA antibodies. Ultimately, the role of circulating cell-free DNA is unclear but it appears that high levels of anti-dsDNA antibodies are a pathogenic response to DNA antigens, highlighting the importance of DNASEs to clear circulating debris containing nucleic acids.

Figure 1: Presence of cell-free DNA in the serum of patients with SLE. Double diffusion assay in agarose gel was used to detect anti-DNA antibodies and cell-free DNA in SLE sera. Peripheral wells contained SLE patient sera 1–6. Central well A contained calf thymus DNA and central well B contained serum from another SLE patient. Sera 2 and 3 precipitated with thymus DNA, indicating these sera contained anti-DNA antibodies. Sera 2 and 3 also reacted with SLE serum in well B, suggesting the presence of DNA in SLE serum, which was confirmed by a chemical method (reproduced and modified from Figure 2, Tan et al. with permission; license No. 1165799-1). SLE, systemic lupus erythematosus.

Figure 2: Cell-free DNA in the circulation. Cell-free DNA are released into blood due to cell death including necrosis, apoptosis, neutrophil extracellular net formation (NETosis), and pyroptosis. In addition, live cells also secrete cell-free DNA. DFFB tends to cleave double stranded DNA at internucleosome linker regions in chromatin into higher molecular weight DNA fragments and then into oligonucleosomal fragments. Cell-free DNA fragments are cleared by DNASE1 (for <150 bp fragments) and DNASE1L3 (for larger and chromatin associated DNA). DFFB, DNA fragmentation factor B; NETosis, neutrophil extracellular traps.
**DNASE1L3 Gene Deficient Mice Develop Anti-DNA Antibodies and Lupus-like Disease**

There have been several animal studies to elucidate the underlying pathophysiology of ineffective clearance of cellular debris. DNASES are primarily responsible for lysis and breakdown of DNA both in tissues and circulation. Human and animal models have shown that a lack of DNASES in subjects led to proliferation of local inflammatory response, production of anti-dsDNA antibodies, and ultimately SLE-like disease. DNASE 1, 2, and TREX1/DNASE 3 have been explored, with DNase 1 family being the most implicated.

DNASE 1 endonucleases cleave DNA into 5'-phosphates and 3'-hydroxy ends in a Ca\(^{2+}\)/Mg\(^{2+}\) dependent, Zn\(^{2+}\) sensitive manner.[8] The family of enzymes includes DNASE1, DNASE1L1, DNASE1L2, and DNASE1L3. DNASE1 and DNASE1L3 are active in circulating blood, being predominantly responsible for the breakdown of circulating cell-free DNA (Figure 2). DNASE1L1 is active in skeletal muscle and cardiomyocytes while DNASE1L2 is active in keratinocytes.[9] DNASE1L3 has peak activity in neutral pH and can bind to nucleosomes containing either double stranded or single stranded DNA with chromatin complexes. DNASE1L3 moreover possesses a uniquely positively charged C-terminal that facilitates membrane or protein bound DNA but makes it less efficient at breaking down naked DNA compared to DNASE1L1.[8]

Given this proclivity for DNASE1L3 to bind to DNA and chromatin complexes, it is thought to play a larger role in digesting nucleic acids from self-sources such as apoptosis or NETosis, which often have longer cell-free DNA fragments bound to membrane microparticles or proteins (Figure 2).[10] In recent studies, DNASE1L3 deficient mice had a higher proportion of longer sized circulating cell-free DNA, suggesting that DNASE1L3 was primarily responsible for the breakdown of larger, multinucleosomal sized cell-free DNA into smaller mononucleosomal cell-free DNA.[10] Mice with targeted DNASE1L3 deletion later developed anti-DNA immunoglobulin G (IgG) antibodies with eventual progression to SLE-like disease.[10] Plasma DNA from DNASE1L3 deficient mice was associated with an increased prominence in mean peak sizes corresponding to di-, tri-, and tetranucleosomal sizes.[11, 12] In corresponding mice with another animal study, cell-free DNA in DNASE1L3 deficient mice required CD40L expressing T cells to proliferate extral follicular B cells that were responsible for a rapid anti-dsDNA response. Similar to wild type mice, DNASE1L3 deficient mice that were also CD40L deficient did not develop reactivity to DNA antigens. Extragol follicular T cell differentiation had been previously identified in patients with lupus nephritis and may be responsible for directly interacting with circulating DNA. Type 1 interferon (IFN-1) signaling has moreover been implicated in this process. In the same study, DNASE1L3 and IFN-1 receptor deficient mice showed reduced levels of anti-dsDNA antibodies. IFN-1 producing plasmacytoid dendritic cells facilitated the differentiation of antibody forming cells. C1q moreover helps regulate the same interferons, and deficiency in C1q also has led to autoantibody formation. Merging these pathways, it is evident that CD40L expressing T cells, complements, and IFN-1 producing plasmacytoid dendritic cells can affect the production of anti-dsDNA antibodies in the presence of certain DNA fragments, highlighting the increasingly evident role of DNASE1L3 in preventing such downstream production.[13]

Further analysis into how T and B cells react to circulating DNA fragments can help better understand the downstream formation of autoantibodies. In another animal study, DNASE1L3 deficient mice required CD40L expressing T cells to proliferate extral follicular B cells that were responsible for a rapid anti-dsDNA response. Similar to wild type mice, DNASE1L3 deficient mice that were also CD40L deficient did not develop reactivity to DNA antigens. Extral follicular T cell differentiation had been previously identified in patients with lupus nephritis and may be responsible for directly interacting with circulating DNA. Type 1 interferon (IFN-1) signaling has moreover been implicated in this process. In the same study, DNASE1L3 and IFN-1 receptor deficient mice showed reduced levels of anti-dsDNA antibodies. IFN-1 producing plasmacytoid dendritic cells facilitated the differentiation of antibody forming cells. C1q moreover helps regulate the same interferons, and deficiency in C1q also has led to autoantibody formation. Merging these pathways, it is evident that CD40L expressing T cells, complements, and IFN-1 producing plasmacytoid dendritic cells can affect the production of anti-dsDNA antibodies in the presence of certain DNA fragments, highlighting the increasingly evident role of DNASE1L3 in preventing such downstream production.[13]

Other modalities to disrupt DNASE1L3 activity include epigenetic factors in the formation of anti-DNA response. In another animal study, cell-free DNA in DNASE1L3 deficient
In another report, a 31-year-old with γ-DS DNA autoantibodies and low complement C3 and C4 levels. In another study, the presence of an IFN-γ-dependent membrane microparticle membrane was found to play a role in enveloping circulating DNA fragments that in turn limited immunogenicity of those fragments. In DNASE1L3 deficient mice, this barrier was weaker compared to controls, contributing to the ease of access to membrane-bound DNA to immune cells.\(^{[15]}\)

**DNASE1L3 Gene Null Mutation in Monogenic SLE**

Lupus and lupus-like diseases have been associated with many genetic abnormalities affecting various components of the immune response. One such culprit includes inherited deficiencies in the complement system. Deficiencies in many complements including C1q have been implicated in inherited lupus, which likely contribute to the lack of clearance of apoptotic debris and subsequent self-immune response.\(^{[16]}\) More than 30 genes with over a 100 susceptibility loci have been described.\(^{[17]}\) In one review of 71 patients with SLE and their parents, whole genome sequencing revealed rare missense and nonsense mutations in 22 genes known to cause SLE. Out of these, genetic defects affecting complement function, particularly C1q, were associated with the highest penetrance of SLE.\(^{[18]}\) C1q normally inhibits IFN-1 and other cytokine responses either directly through leukocyte associated Ig-like receptor 1 (LAIR) on dendritic cells or by inhibition of Toll-like receptor (TLR)-7 and TLR9. With this lack of inhibition, plasmacytoid dendritic cells can potentially contribute to differentiation of antibody forming cells.

Other culprit mutations included those in DNASE1 and DNASE1L3. One report identified a lupus-like disease in an 8-year-old with recurrent pulmonary hemorrhage, urticarial rash, hypocomplementemia, and glomerulonephritis. Whole-exome sequencing revealed a DNASE1L3 gene frameshift and premature truncation mutation. Parents were heterozygous for the same mutation. In another cohort studies, single-nucleotide polymorphisms of DNASE1 were associated with risk of SLE but did not correlate directly with DNASE activity.\(^{[19, 20]}\) In another report, a 31-year-old with an inherited homozygous deletion in DNASE1L3 exhibited a lupus-like syndrome with a hypocomplementemic vasculitis.\(^{[21]}\) Al-Mayouf et al.\(^{[22]}\) reported that loss-of-function variant in DNASE1L3 causes familial form of SLE. Sequencing DNASE1L3 uncovered a homozygous 1-bp deletion in DNASE1L3 (c643delT). Multimembers of 7 families developed SLE at an early age with anti-dsDNA antibody production along with low complement C3 and C4 levels. In another report, 2 families with 5 affected children were identified to have homozygous frameshift mutation, c.289_290delAC and exon skipping, c.320+4delAGTA.\(^{[23]}\) These affected children developed hypocomplementemic urticarial vasculitis (HUVS) syndrome and 4 out of 5 developed SLE with anti-dsDNA antibody production.

Isolated DNASE deficiency is rare but the evident association with SLE and SLE-like disease highlights the role of the DNASE 1 family of endonucleases, particularly of DNASE1L3, in the pathogenesis of SLE. Identifying the mechanism of formation of DNA substrates and function of DNASES can further elucidate the significance of such deficiencies in SLE.

**Impaired DNASE1L3 in Patients with SLE**

Decreased protein levels and enzymatic activity of DNASE1L3 have been reported in patients with SLE and those with dermatomyositis/polymyositis.\(^{[24, 25]}\) In SLE patients, DNASE1L3 protein levels inversely correlate with the presence of anti-DNA autoantibodies and renal involvement.\(^{[26]}\) Factors responsible for reduced DNASE1L3 activity can result from DNASE1L3 mutations and/or the presence of DNASE1L3 inhibitors (Figure 3). DNASE1L3 null mutation leads to absence of DNASE1L3 and development of lupus in early life (as described above). However, DNASE1L3 null mutation is rare in sporadic lupus. Thus, it is likely that a majority of sporadic lupus patients will not carry DNASE1L3 null mutations. DNASE1L3 rs35677470 polymorphism results in R206C mutation, which is associated with development of lupus.\(^{[26, 27]}\) Previous studies reported that R206C mutation results in the elimination of DNASE1L3 enzymatic activity.\(^{[28, 29]}\) Structural analysis predicted that this single nucleotide polymorphism

![Figure 3: Impaired DNASE1L3 activity contributes to production of anti-DNA autoantibodies in SLE. Impaired DNASE1L3 activity results from deficiency or mutation of DNASE1L3 gene or presence of anti-DNASE1L3 autoantibodies. Absence of DNASE1L3 or its reduced activity leads to accumulation of chromatin associated long DNA fragments and anti-DNA autoantibody production. DNASE1L3, deoxyribonuclease 1 like 3; SLE, systemic lupus erythematosus.](image-url)
(SNP) causes an inactive form of DNASE1L3. However, these notions are inconsistent with the phenotypes of sporadic lupus, since absence of DNASE1L3 results in monogenic pediatric lupus. In fact, R206C causes impaired secretion of DNASE1L3 protein, but its enzymatic activity remains intact. These were demonstrated elegantly by in-vitro experiments that compared levels of DNASE1L3 protein expression by variant 206R (arginine) versus 206C (cysteine). 206C variant DNASE1L3 exhibited a substantially reduced level in the supernatant of human embryonic kidney (HEK) cells transfected with cDNA encoding 206C, but accumulation of DNASE1L3 intracellularly in HEK cells. However, the 206C variant of DNASE1L3 retains the enzymatic activity to digest chromatic DNA. These findings are consistent with the clinical phenotype in those of SLE carrying this risk SNP. R206C appears to be Caucasian-specific. Though less frequent, a non-synonymous SNP, G82R (rs73450392), is also shown to diminish the enzymatic activity of DNASE1L3 and further may contribute to the production of anti-DNA antibodies in SLE.

Neutralizing Autoantibodies to DNASE1L3 in Patients with SLE

Impairment of NETosis remnant clearance has been associated with lupus nephritis. One of the mechanisms responsible for the impairment was suspected to be the presence of anti-DNASE antibodies. This hypothesis was further strengthened by the observation that decreased DNASE1L3 activity in lupus patients was restored by removal of serum IgG using protein A, suggesting the presence of anti-DNASE1L3 antibodies in the circulation of lupus patients.

Indeed, autoantibodies to DNASE1L3 have been described. In a serological analysis of SLE patients with renal involvement, which involved a population known to have high levels of pathogenic anti-dsDNA levels, >50% of patients in that cohort exhibited reduced DNASE1L3 activity in circulation compared to healthy controls or SLE patients without renal disease. Ultimately, those patients were found to have increased levels of anti-DNASE1L3 antibodies that led to decreased DNASE1L3 activity. The resultant effects were similar to those seen in patients and animal models with DNASE1L3 deficiency – increased levels of longer circulating DNA fragments and microparticles containing complexes of protein or membrane-bound DNA. The presence of anti-DNASE1L3 antibodies correlated with decreased enzymatic activity of DNASE1L3, indicating a neutralizing effect.

Conclusions

The presence of anti-dsDNA antibodies has been strongly linked to the development of SLE and related organ injury. Impairment of DNASE1L3 activity has been implicated in animal and human studies in the development of these antibodies due to the role of DNASE1L3 in the clearance of circulating long protein and membrane-bound cell-free DNA fragments. Multiple modalities of defective DNASE1L3 have been described, from monogenic inherited deficiencies to formation of antibodies to DNASE1L3. Resultant long and short nucleic acid fragments in the setting of DNASE1L3 deficiency can elicit a cytokine and associated T cell response to rapidly promote autoreactive B cells to form anti-dsDNA antibodies. This highlights the integral role of circulating DNASE1L3 in maintaining self-tolerance and preventing progression of autoimmunity in SLE.

References

[1] Ceppellini R, Polli E, Celada F. A DNA-Reacting Factor in Serum of a Patient with Lupus Erythematosus Diffusus. Proc Soc Exp Biol Med. 1957;96(3):572–574.
[2] Tan EM, Schur PH, Carr RI, et al. Deoxybonucleic Acid (DNA) and Antibodies to DNA in the Serum of Patients with Systemic Lupus Erythematosus. J Clin Invest. 1966;45(11):1732–1740.
[3] Rekvig OP. The dsDNA, Anti-dsDNA Antibody, and Lupus Nephritis: What We Agree on, What Must Be Done, and What the Best Strategy Forward Could Be. Front Immunol. 2019; 10: 1104.
[4] Soni C, Reizis B. DNA as a Self-Antigen: Nature and Regulation. Curr Opin Immunol. 2018;55: 31–37.
[5] Jiang P, Xie T, Ding SC, et al. Detection and Characterization of Jagged Ends of Double-Stranded DNA in Plasma. Genome Res.
Loss-of-Function New Horizons in Whole-Genome Digestion of Chromatin Origin and Anti-Tumor Effects of Association of DNASE1L3 Caucasian-Specific Plasma DNA Profile Nuclease Deficiencies Amyloid-DNA An Autosomal Dnase1l3 Deficiency Recessive DNASE1L3-Related Autoimmune Disease with Unusual [21] Carbonella A, Mancano G, Gremese E, et al. An Autosomal Recessive DNASE1L3-Related Autoimmune Disease with Unusual Clinical Presentation Mimicking Systemic Lupus Erythematosus. Lupus, 2017;26(7):768–772.

[22] Al-Mayouf SM, Sunker A, Abdwani R, et al. Loss-of-Function Variant in DNASE1L3 Causes a Familial Form of Systemic Lupus Erythematosus. Nat Genet. 2011;43(12):1186–1188.

[23] Özşaker ZB, Foster J, 2nd, Díaz-Horta O, et al. DNASE1L3 Mutations in Hypocomplementemic Urticarial Vasculitis Syndrome. Arthritis Rheum. 2013;65(8):2183–2189.

[24] Bruschi M, Bonanni A, Petretto A, et al. Neutrophil Extracellular Traps Profiles in Patients with Incident Systemic Lupus Erythematosus and Lupus Nephritis. J Rheumatol. 2020;47(3):377–386.

[25] Zhao Q, Yang C, Wang J, et al. Serum Level of DNase1l3 in Patients with Dermatomyositis/Polyarthritis, Systemic Lupus Erythematosus and Rheumatoid Arthritis, and its Association with Disease Activity. Clin Exp Med. 2017;17(4):459–465.

[26] Gateva V, Sandling JK, Hom G, et al. A Large-Scale Replication Study Identifies TNIP1, PRDM1, JAZF1, UHRF1BP1 and IL10 as Risk Loci for Systemic Lupus Erythematosus. Nat Genet. 2009;41(11):1228–1233.

[27] International Consortium for Systemic Lupus Erythematosus Genetics (SLEGEN); Harley JB, Alarcon-Riquelme ME, et al. Genome-Wide Association Scan in Women with Systemic Lupus Erythematosus Identifies Susceptibility Variants in ITGAM, PXK, KIAA1542 and Other Loci. Nat Genet. 2008;40(2):204–210.

[28] Ueki M, Takeshita H, Fujihara J, et al. Caucasian-Specific Allele in Non-Synonymous Single Nucleotide Polymorphisms of the Gene Encoding Deoxyribonuclease I-like 3, Potentially Relevant to Autoimmunity, Produces an Inactive Enzyme. Clin Chim Acta. 2009;407(1–2):20–24.

[29] Zervou MI, Andreou A, Matalliotakis M, et al. Association of the DNASE1L3 rs35677470 Polymorphism with Systemic Lupus Erythematosus, Rheumatoid Arthritis and Systemic Sclerosis: Structural Biological Insights. Mol Med Rep. 2020;22(6):4492–4498.

[30] Coke LN, Wen H, Comeau M, et al. Arg206Cys Substitution in DNASE1L3 Causes a Familial Form of Systemic Lupus Erythematosus. Ann Rheum Dis. 2021;80(6):782–787.

[31] Bruschi M, Bonanni A, Petretto A, et al. Neutrophil Extracellular Trap Degradation is Associated with Lupus Nephritis. Proc Natl Acad Sci U S A, 2012;109(21):8913–8918.

[32] Leffler J, Martin M, Gullstrand B, et al. Impairment of Neutrophil Extracellular Traps that are not Degraded in Systemic Lupus Erythematosus Activate Complement Exacerbating the Disease. J Immunol. 2012;188(7):3522–3531.

[33] Hart J, Serpas L, Wang Y, et al. Autoantibody-Mediated Impairment of DNASE1L3 Activity in Sporadic Systemic Lupus Erythematosus. J Exp Med. 2021;218(5):e20201138.