OPINION

Structural modification of DNA—a therapeutic option in SLE?

Steffen Frese and Betty Diamond

Abstract | Systemic lupus erythematosus (SLE) is an autoimmune disease that affects multiple organs, with glomerulonephritis representing a frequent and serious manifestation. SLE is characterized by the presence of various autoantibodies, including anti-DNA antibodies that occur in approximately 70% of patients with SLE and which contribute to disease pathogenesis. Consequently, immunosuppressive therapies are applied in the treatment of SLE to reduce autoantibody levels. However, increasing evidence suggests that DNA—especially double-stranded DNA—constitutes an important pathogenic factor that is able to activate inflammatory responses by itself in autoimmune diseases. Therefore, modifying the structure of DNA to reduce its pathogenicity might be a more targeted approach for the treatment of SLE than immunosuppression. This article presents information in support of this strategy, and discusses the potential methods of DNA structure manipulation—in light of data obtained from mouse models of SLE—including topoisomerase I inhibition, administration of DNase I, or modification of histones using heparin or histone deacetylase inhibitors.

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Introduction

Systemic lupus erythematosus (SLE) is a chronic autoimmune disease that can affect the cardiac, pulmonary and central nervous systems, as well as the skin, joints, and kidneys. Involvement of kidneys, in the form of glomerulonephritis (lupus nephritis), contributes considerably to the morbidity and mortality of patients with SLE. Although an area of intense research, the etiology of SLE is not fully understood. The disease is characterized by the presence of various autoantibodies, including those directed against double-stranded DNA (dsDNA) and other nuclear antigens. A hypothesis now widely accepted is that these autoantibodies are generated by a misguided immune response to apoptotic cells or cells undergoing secondary necrosis, which can follow apoptotic cell death. Under normal conditions, genomic DNA is not accessible to the immune system; however, when cells die by apoptosis, apoptotic bodies containing fragmented cellular structures and cleaved genomic DNA are released, enabling the immune system to access DNA and/or other nuclear antigens.[1] Mechanisms exist to ensure that this source of antigenic material is not immunogenic under normal conditions. Although the exact mechanisms that drive the generation of pathogenic autoantibodies in patients with SLE remain to be determined, it seems that defective clearance of apoptotic cells,[2] the length of DNA fragments,[3] and modifications to DNA such as hypomethylation[4] can all contribute to this process. The major nuclear antigen in SLE seems to be the nucleosome, which is made up of dsDNA bound to the five histone molecules, H1, H2A, H2B, H3 and H4, and antigenic DNA is most likely to be present in this form in patients with the disease.

Currently, therapies routinely used for the treatment of SLE, or that are in development, aim to reduce the production of autoantibodies by direct suppression of B cells or through general immunosuppression; however, many of the agents used are associated with severe adverse events or have yet to show clinical efficiency in patients with active lupus nephritis. Therefore, treatment strategies besides immunosuppression should be actively sought. In this context, this article discusses DNA as a pathogenic factor in SLE, and potential approaches that could be taken to directly manipulate the structure of DNA and reduce this pathogenicity are introduced.

Competing interests
S. Frese declares that he is a patent applicant. See the article online for full details. B. Diamond declares no competing interests.

DNA–antibody-induced damage

The presence of anti-dsDNA antibodies in patients with SLE was demonstrated more than 60 years ago. A large body of evidence suggests that these autoantibodies are a major contributor to the pathogenesis of SLE. A broadly accepted scenario for the mechanism of antinuclear-antibody-initiated damage in this disease is based on the observation that kidneys of mice and humans with SLE-associated glomerulonephritis contain immune complexes composed of anti-dsDNA antibodies and nucleosomal DNA (Figure 1). Moreover, nucleosomal DNA bound to IgG can be detected both in the sera of human patients with SLE and in mice with SLE-like disease. Whether immune complexes are built up in the circulation and subsequently bind to certain structures of the kidney such as the glomerular basement membrane (GBM) and mesangial matrix, or whether anti-dsDNA antibodies form a complex with DNA already bound to glomerular structures is not clear at present. After binding to the kidney, however, immune complexes are able to activate complement[5] and Fc-receptor-bearing effector cells,[6] thus initiating an inflammatory reaction.

DNA-sensing pathways

The mechanisms involved in the development of SLE-associated glomerulonephritis that we have describe place anti-dsDNA antibodies at center stage; however, growing evidence suggests that under autoimmune conditions, DNA fragments alone are able to induce signaling cascades that promote inflammation. Much of the data were originally derived from studies using viral or bacterial nucleic acids, which are known to initiate an inflammatory response mediated by the innate immune system. Meanwhile,

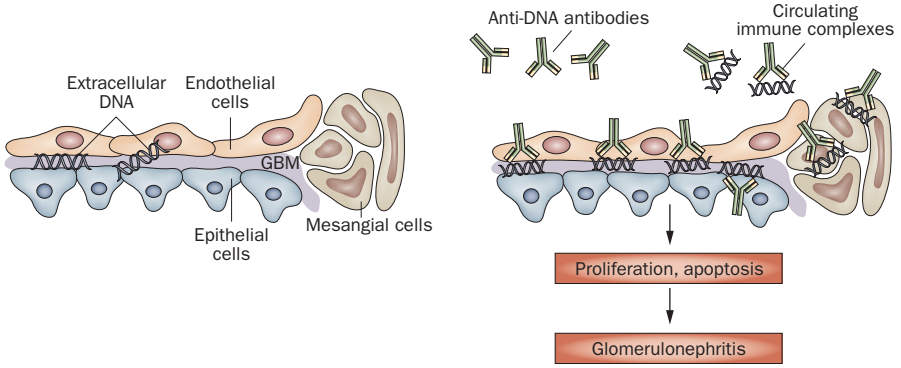

Figure 1 | Induction of glomerulonephritis by SLE-associated anti-dsDNA antibodies. Anti-dsDNA antibodies are able to bind to extracellular DNA, which itself can bind to GBMs. The resulting deposition of immune complexes in the kidneys leads to the recruitment and activation of complement proteins and cells bearing FcR, which promote inflammatory reactions. Whether anti-dsDNA antibodies recognize DNA already bound to GBMs or whether pre-existing DNA–anti-dsDNA antibody complexes deposit in kidneys remains unclear. Abbreviations: dsDNA, double-stranded DNA; FcR, Fc receptor; GBM, glomerular basement membrane; SLE, systemic lupus erythematosus.

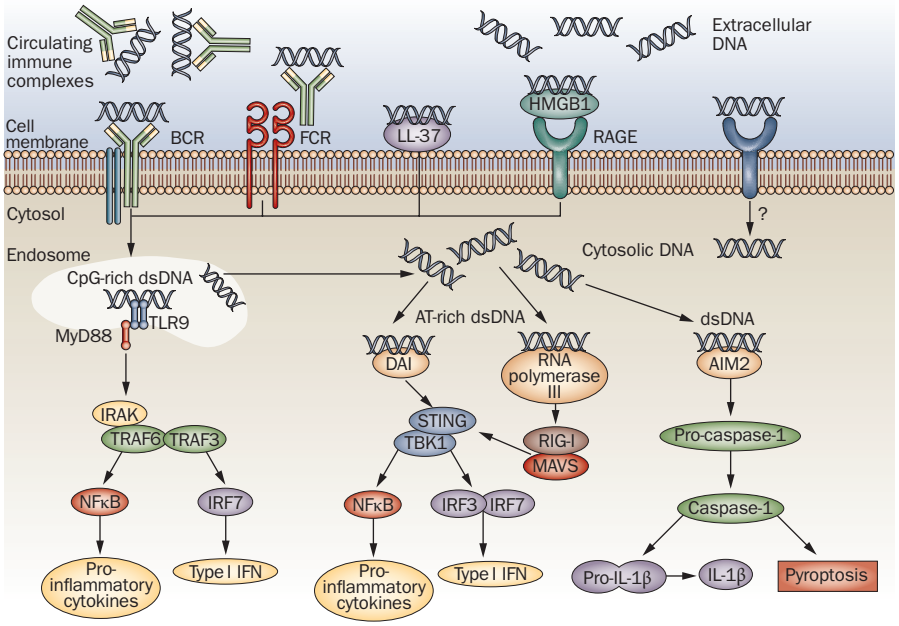

Figure 2 | Pathways involved in internalization and sensing of dsDNA. dsDNA-containing immune complexes undergo endocytosis after engaging the BCR on B cells or FcRs on DCs, macrophages and glomerular cells. Additionally, dsDNA can be internalized after binding to LL-37, or through the HMGB1–RAGE pathway. These routes result in localization of DNA to endosomes. It is not known if DNA is released from endosomes to the cytoplasm; additional receptors that mediate direct uptake into the cytoplasm might exist. The dsDNA-sensing pathways activated differ according to the structure of the DNA. CpG-rich dsDNA activates TLR9 whereas AT-rich dsDNA signals through DAI or RNA polymerase III. These signaling pathways all lead to production of type I IFN and inflammation. A fourth dsDNA-sensing pathway involves the AIM2 inflammasome, and results in activation of IL-1β and induction of pyroptosis. Abbreviations: BCR, B-cell receptor; DCs, dendritic cells; dsDNA, double-stranded DNA; FcR, Fc receptor; IFN, interferon; IRF, interferon regulatory factor; NFκB, nuclear factor κB.

pathways through which self DNA is able to induce proinflammatory reactions have been identified that are distinct from those activated by microbial nucleic acids. A shared feature of these pathways is the resultant production of type I interferons (IFNs) and other proinflammatory cytokines. Interestingly, only dsDNA has the ability to stimulate these signaling pathways, as demonstrated in 1999 by Suzuki *et al.*,[7] who discovered that only double-stranded and not single-stranded polynucleotides increase type I IFN-dependent expression of MHC complex proteins. The following sections summarize the dsDNA-sensing pathways that have been identified to date.

The Toll-like receptor 9 pathway

The specific signaling cascade that is activated by dsDNA depends on the cell type and on the structure of the DNA. Unmethylated CpG-rich dsDNA has been shown to be a ligand for Toll-like receptor (TLR) 9, a receptor localized to endosomes. Binding of unmethylated CpG-rich dsDNA to TLR9 activates the adaptor protein myeloid differentiation primary response protein MyD88 and, subsequently, interferon regulatory factor (IRF) 7 as well as nuclear factor κB (NFκB), resulting in the production of type I IFNs and other proinflammatory cytokines (Figure 2).[8] Investigations into the role of TLR9 signaling in the pathogenesis of SLE that used mice prone to SLE-like disease have produced conflicting results. In contrast to studies performed in NZB/W F1 mice, in which TLR9 activation was shown to contribute to the pathogenesis of lupus nephritis,[9] signaling through TLR9 seems to have a protective role in mice possessing the MRL^{lpr} genetic background.[10] The latter effect might be explained by data presented in a 2011 publication by Stoehr *et al.*,[11] which demonstrate a contribution of TLR9 signaling to the production of protective IgM antibodies. In this report, the authors describe their observation that double-knockout of both the inhibitory Fc receptor FcγRIIB (low affinity immunoglobulin γ Fc region receptor II, also known as CD32) and TLR9 in mice exacerbated lupus-like disease, which was accompanied by low peritoneal B-1b cell numbers, decreased levels of self-reactive IgM autoantibodies, and by the accumulation of proinflammatory type 17 T helper (T_H17) cells. Transfer of TLR9-expressing peritoneal B-1b cells or recombinant IgM antibodies into these mice decreased the number of T_H17 cells and ameliorated lupus-like disease,[11] suggesting a role for TLR9-expressing peritoneal B-1b cells in maintenance of self-tolerance.

Sensing pathways for AT-rich dsDNA

In addition to activation of TLR9 mediated by CpG-rich dsDNA, at least three other TLR-independent DNA-sensing signaling pathways have been identified. Two of these pathways are induced by AT-rich (B-form or non-CpG-rich) dsDNA. Once AT-rich dsDNA is internalized into the cytoplasm, a cascade involving the signaling proteins

stimulator of interferon genes (STING, also known as transmembrane protein 173) and TANK-binding kinase 1 (TBK1) as well as the transcription factors IRF3, IRF7 and NFκB is activated, resulting in the production of type I IFNs and other proinflammatory cytokines.[12,13] Two cytosolic pathways that can initiate this AT-rich dsDNA-dependant response have been identified. The first is mediated by the DNA-dependent activator of IRF (DAI, also known as DLM-1 or ZBP1),[14] and the second is characterized by the involvement of RNA polymerase III.[15] Binding of AT-rich dsDNA to RNA polymerase III produces transcripts of double-stranded RNA that can activate the retinoic acid-induced gene I (RIG-I) and mitochondrial antiviral signaling protein (MAVS), finally resulting in the production of type I IFNs (Figure 2).[15] Thus, at least three different dsDNA-sensing pathways encompassing TLR9, DAI and RNA polymerase III exist that are able to induce type I IFN production, and, consequently, promote inflammatory responses.

Inflammasome-dependent DNA sensing

In 2008, Muruve *et al.*[16] reported the identification of an additional signaling cascade involved in sensing of cytoplasmic dsDNA. This pathway is activated after binding of cytoplasmic dsDNA to the AIM2 inflammasome complex consisting of the HIN200 domain-containing protein AIM2, the ASC adaptor protein and caspase-1. This binding leads to subsequent activation of IL-1β and pyroptosis—a proinflammatory type of cell death (Figure 2). Importantly, as shown for other DNA-sensing mechanisms, only dsDNA and not single-stranded DNA was able to induce the AIM2-inflammasome-dependent signaling pathway.[16,17]

TLR-independent DNA sensing and SLE

The relevance of TLR-independent DNA sensing to the pathogenesis of SLE has been demonstrated in several experimental studies. Purified nucleosomal DNA, which is found in the serum of patients with SLE as well as mice with SLE-like disease, was shown to activate dendritic cells (DCs) obtained from normal and MyD88-deficient mice, suggesting TLR-independence.[18] This effect in DCs might explain some of the SLE-associated hyperactivation of the immune system; however, TLR-independent sensing of dsDNA also occurs in disease-affected organs such as kidney, where it has been demonstrated in both glomerular mesangial cells and endothelial cells. Interestingly, treatment of glomerular endothelial monolayers with AT-rich dsDNA increased their permeability to albumin, suggesting that DNA sensing by glomerular cells might indeed contribute to the proteinuria associated with lupus nephritis.[19,20]

Uptake of extracellular DNA

Antibody-dependent uptake

A pivotal question is how extracellular dsDNA enters immune cells and/or cells of organs affected by the disease. Several studies have highlighted the fact that different routes of entry are available depending on whether DNA is bound to autoantibodies in circulating immune complexes or exists as unbound extracellular dsDNA within the nucleosome. DNA-containing immune complexes present in the circulation can be endocytosed after engagement of B-cell receptors on B cells,[21] or FcγRIIB on DCs (Figure 2).[22] Interestingly, initiation of cell-activating signaling pathways by SLE-derived dsDNA–antibody complexes, but not normal IgGs, was shown to involve engagement of either the B-cell receptor or FcγRIIB together with TLR9.[21,22]

Antibody-independent uptake

In contrast to circulating DNA-containing immune complexes, dsDNA not bound to autoantibodies can be internalized by at least two distinct, FcR-independent pathways. One involves the antimicrobial peptide LL-37,[23] and the other is dependent on the DNA-binding protein high-mobility group protein B1 (HMGB1) and the receptor for advanced glycation end products (RAGE)—a member of the immunoglobulin superfamily (Figure 2).[24] Internalization and sensing of dsDNA not bound to autoantibodies probably plays a part in the pathogenesis of SLE, as supported by data demonstrating that DC maturation in mice occurs after exposure to purified nucleosomes.[18] Moreover, the double-stranded structure of DNA seems to be important, not only for sensing but also for internalization, as only class A and not class B CpG oligodeoxynucleotides (which are maintained in single-stranded forms) were able to bind HMGB1.[24]

The four pathways so far identified as being involved in the cellular uptake of extracellular dsDNA do not explain how the endocytosed DNA reaches the cytoplasm, because internalization by immunoglobulin receptors as well as by LL-37 have been shown to localize the DNA to endosomes. An answer to the question of how DNA reaches the cytoplasm might be derived from the postulated existence of additional, as yet unidentified, receptors that mediate the uptake of extracellular DNA directly into the cytoplasm. Alternatively, it might be that dsDNA reaches the cytoplasm via an indirect route; indeed, some data have been presented that demonstrate the release of nucleic acids from endosomes to the cytoplasm (Figure 2).[25]

Manipulation of DNA structure

As demonstrated by the studies we have described, DNA has a key role in the pathogenesis of SLE; therefore, research into this disease might be advanced by paying more attention to DNA itself as a therapeutic target. Theoretically, at least two different strategies are possible, both of which rely on manipulation of the structure of DNA. Firstly, manipulation of extracellular nucleosomal DNA could prevent the binding of anti-dsDNA antibodies and, therefore, avert the subsequent immune-complex-dependent tissue damage and immune activation. Secondly, changing the structure of extracellular DNA, regardless of its binding to autoantibodies, might reduce the internalization and sensing of pathogenic oligonucleotides. As these processes can result in initiation of signaling pathways that lead to profound activation of various immune responses, inhibition of sensing should reduce the inflammatory effects of extracellular DNA. Increasing evidence suggests that therapies which take either of these approaches to reducing the pathogenicity of extracellular DNA could have a positive impact on the treatment of SLE. The following sections outline the methods of DNA and/or nucleosome structure manipulation that have shown therapeutic potential *in vitro* and in animal models of SLE-like disease.

Nucleosome modification by heparin

Treatment of patients with the glucosaminoglycan heparin or its derivates might be one possible approach to structural modification of nucleosomal DNA. The heparin molecule is highly negatively charged, which enables it to bind to the positively charged histone proteins that mediate the packaging of DNA into nucleosomes. Heparin reduced the association of nucleosome–anti-nucleosome antibody complexes with the GBM proteins heparan sulfate,[26] laminin and collagen[27] *in vitro,* and, in turn, diminished the binding of these immune complexes to intact GBMs *in vivo*.[26,27] The decreased binding of nucleosomal DNA to GBMs was attributed to heparin-induced changes in the charge

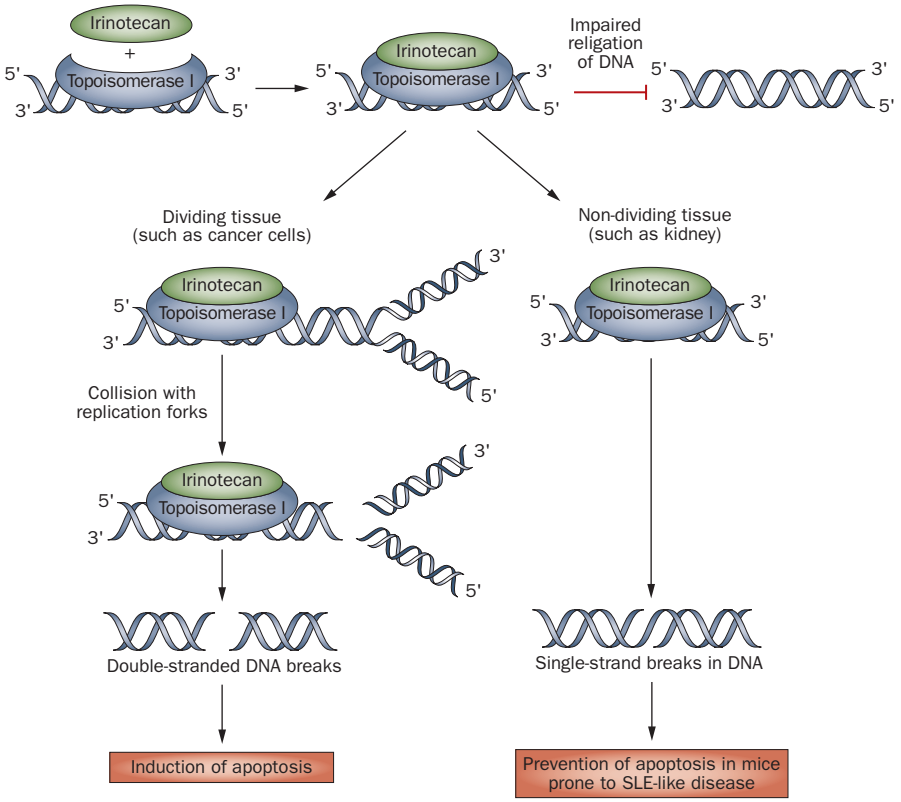

Figure 3 | Cellular effects of topoisomerase I inhibitors in dividing and non-dividing cells. Topoisomerase I binds dsDNA and induces single-strand breaks to relieve torsional stress. Binding of the topoisomerase I inhibitor irinotecan prevents topoisomerase I-mediated religation of the DNA. The complex containing DNA, topoisomerase I and irinotecan can collide with replication folks, which exist only in rapidly proliferating cells, resulting in double-strand breaks in DNA and subsequent induction of apoptosis. In non-dividing tissue, such as kidney, irinotecan causes single-strand DNA breaks, which are not lethal to the cell. In fact, irinotecan treatment seems to prevent apoptosis and ameliorate lupus nephritis in a mouse model of spontaneous SLE. Abbreviations: dsDNA, double-stranded DNA; SLE, systemic lupus erythematosus.

on nucleosomal histones and/or changes in conformation of the nucleosomal structure. Indeed, electrostatic forces are important for histone-mediated packaging of DNA —neutralization of the charge on these proteins by heparin would reduce their interaction with DNA, and could change the structure of the nucleosome. Importantly, recognition of nucleosomal DNA by anti-dsDNA antibodies was not affected by heparin. This finding suggests that the reduction in glomerular antibody deposition was a direct result of decreased binding of nucleosomes to GBMs—which are negatively charged—and was not caused by abrogation of nucleosome-containing immune complex formation. Additionally, nucleosomes incubated with heparin were found to be more susceptible to digestion by the endonuclease deoxyribonuclease (DNase) I *in vitro*,[27,28] presumably as a consequence of heparin-induced modification of the nucleosome structure that increased the accessibility of nucleosomal DNA to this enzyme.

Evidence derived from animal models of SLE-like disease support the therapeutic potential of heparin highlighted by the data described above. For example, treatment of MRL^{lpr} and NZB/W F1 mice with heparin was shown to decrease autoantibody production and to ameliorate lupus nephritis.[26,27] However, from the clinical point of view, it has to be considered that some heparins have anticoagulative activity; therefore, clinical trials using heparin for the treatment of SLE must exhibit benefit at doses that minimally affect blood clotting.

Histone deacetylase inhibitors

Histone deacetylase (HDAC) inhibitors (HDIs) might represent an alternative to heparin for structural modification of nucleosomal DNA. Acetylation of the histone proteins is a strictly regulated endogenous cellular process, which is known to mediate changes in nucleosomal structure. Addition of acetyl groups to specific positively charged amino acids in the histones has a similar charge neutralization effect to heparin binding. Thus, inhibition of HDACs, which remove the acetyl groups, results in accumulation of acetylated histones and alterations in the structure of chromatin. In mice prone to SLE-like disease, the HDI trichostatin A reduced the levels of proteinuria and glomerular deposition of immune complexes in comparison with vehicle-treated controls.[29] Although the authors of this study suggested that a trichostatin A-induced increase in CD4$^+$ CD25$^+$ regulatory T cells was responsible for the demonstrated effects, no data were shown to support this hypothesis. In fact, the combined application of trichostatin A and cell depleting anti-CD25 antibodies did not prevent the beneficial effects of the HDI.[29] An alternative explanation for the trichostatin A-mediated suppression of lupus nephritis could be that increased acetylation of nucleosomal histones decreased nucleosome binding to GBMs, which would prevent subsequent glomerular accumulation of anti-DNA antibodies.

Inhibitors of topoisomerase I

In addition to the modification of nucleosomal histones, the generation of single-strand breaks in DNA by inhibitors of topoisomerase I might be another approach to targeting DNA in the treatment of SLE. Topoisomerase I is a ubiquitously expressed enzyme responsible for reducing the torsional stress that develops during replication of DNA. To affect this result, topoisomerase I induces a transient single-strand break in the DNA, which is followed by stress-relieving rotation. Next, topoisomerase I religates the single-strand break to reconstitute intact dsDNA. Inhibitors of topoisomerase I such as irinotecan bind to the complex containing topoisomerase I and DNA, also named the 'cleavable complex'. Binding of topoisomerase I inhibitors to this complex can have two possible consequences. If the cell is in S-phase of the cell cycle —during which enhanced DNA replication occurs—the complex comprising inhibitor, topoisomerase I and DNA can collide with replication forks, generating irreversible double-strand breaks in the DNA that would result in the induction of apoptosis (Figure 3).[30] This effect of topoisomerase I inhibitors occurs only in rapidly dividing cells such as cancer cells, and explains why irinotecan is used as a chemotherapeutic agent. A different outcome is possible in non-dividing cells (such as renal cells) where inhibition of DNA-bound topoisomerase I

prevents the religation of genomic DNA, resulting in the production of single-strand breaks in the DNA double helix. Importantly, the accelerated production of single-strand breaks in DNA is not lethal to the cell, but reduces its capacity to replicate (Figure 3).[31]

Our group showed recently that the topoisomerase I inhibitor irinotecan efficiently suppresses glomerulonephritis in NZB/W F1 mice.[32] The levels of anti-dsDNA antibodies and total serum IgG were not reduced in irinotecan-treated mice compared to saline-treated mice, suggesting that irinotecan affected the kidney tissue directly rather than acting through suppression of the immune system. We found a complete attenuation of subendothelial IgG deposits in the kidneys in irinotecan-treated mice whereas mesangial IgG deposits remained unchanged compared to saline-treated mice, suggesting that irinotecan treatment prevented disruption of GBMs. Single-strand breaks in DNA were detectable immunohistochemically in the kidneys of irinotecan-treated mice, and were associated with a marked inhibition of renal cell apoptosis as determined by measurement of TUNEL staining and caspase-3 activity. We hypothesized that, as the levels of pathogenic anti-dsDNA antibodies did not change, the irinotecan-mediated induction of single-strand breaks in DNA protected kidneys from glomerulonephritis by reducing the binding of extracellular nucleosomal DNA to these antibodies. It has been known for 35 years that antinuclear antibodies derived from patients with SLE bind less efficiently to dsDNA containing single-stranded regions than to dsDNA without single-strand breaks.[33] Alternatively, because only dsDNA and not single-stranded DNA has the ability to initiate DNA-sensing pathways,[7,24] it might also be possible that irinotecan-induced single-strand breaks in DNA protected mice from the proinflammatory effects of DNA sensing. Notably, prevention of undesirable DNA-sensing pathway activation would be expected to affect both immune and glomerular cells.

DNA manipulation using DNase I

In the light of data presented above, one might ask whether there are other biochemical processes that could be exploited to modify the structure of DNA and to protect against the development of lupus nephritis. A potential candidate is presented by the enzyme DNase I. DNase I, in contrast to other endonucleases, is secreted into the extracellular space and is, therefore, found in the blood and gastrointestinal tract. Accordingly, its primary function has been presumed to be the digestion of extracellular DNA.[34] The enzymatic activity of DNase I is reported to be dependant on a defined cationic environment; in the presence of Mg^{2+} and Ca^{2+} the enzyme produced single-strand cuts in the DNA double helix, whereas in the presence of Mn^{2+} and Co^{2+} it made double-strand breaks.[35] These properties of DNase I suggest that it might be a promising therapeutic target in SLE.

Interestingly, DNase I itself has been implicated in the pathogenesis of SLE owing to the observation that serum levels of this enzyme are decreased in patients with the disease.[36] In addition, diminished transcription and decreased protein expression of renal DNase I were demonstrated in mice prone to SLE-like disease.[37] The association between reduced levels of DNase I and SLE indicated that administration of this enzyme might lead to an amelioration of lupus nephritis through destruction of extracellular chromatin and by reducing the level of circulating nucleosomes. However, application of recombinant mouse DNase I to NZB/W F1 mice produced conflicting results. Although the group of Peter Lachmann recorded prolonged survival and an amelioration of histological changes in kidneys in these animals,[38] Verthelyi and colleagues[39] reported no effect of DNase I treatment in the same model. Beneficial effects of DNase I treatment have been demonstrated using an estrogen-induced model of SLE in R4A transgenic mice.[40] When these mice were treated with DNase I at the same time as estradiol, the number of high-affinity DNA-reactive B cells was reduced and immune complex deposition in the kidneys was diminished. This effect was accompanied by a decreased production of type I IFNs by splenic DCs.

Conclusion

The immunosuppressive therapies commonly used for treatment of SLE with active glomerulonephritis are neither sufficiently efficacious nor satisfactorily nontoxic. Alternative immunosuppressive therapies that are in development, such as the use of the proteasome inhibitor bortezomib for depletion of plasma cells,[41] have yet to show clinical efficiency in patients with SLE. Therefore, treatment strategies besides immunosuppression should be actively sought. The data presented within this article suggest that modifying the structure of DNA to reduce its pathogenicity might represent an innovative approach to fight SLE.

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1. Casciola-Rosen, L. A., Anhalt, G. & Rosen, A. Autoantigens targeted in systemic lupus erythematosus are clustered in two populations of surface structures on apoptotic keratinocytes. *J. Exp. Med.* **179**, 1317–1330 (1994).
2. Botto, M. *et al.* Homozygous C1q deficiency causes glomerulonephritis associated with multiple apoptotic bodies. *Nat. Genet.* **19**, 56–59 (1998).
3. Zykova, S. N., Seredkina, N., Benjaminsen, J. & Rekvig, O. P. Reduced fragmentation of apoptotic chromatin is associated with nephritis in lupus-prone (NZB × NZW)F(1) mice. *Arthritis Rheum.* **58**, 813–825 (2008).
4. Wen, Z. K. *et al.* DNA hypomethylation is crucial for apoptotic DNA to induce systemic lupus erythematosus-like autoimmune disease in SLE-non-susceptible mice. *Rheumatology (Oxford)* **46**, 1796–1803 (2007).
5. Unanue, E. & Dixon, F. J. Experimental glomerulonephritis. IV. Participation of complement in nephrotoxic nephritis. *J. Exp. Med.* **119**, 965–982 (1964).
6. Sylvestre, D. L. & Ravetch, J. V. Fc receptors initiate the Arthus reaction: redefining the inflammatory cascade. *Science* **265**, 1095–1098 (1994).
7. Suzuki, K. *et al.* Activation of target-tissue immune-recognition molecules by double-stranded polynucleotides. *Proc. Natl Acad. Sci. USA* **96**, 2285–2290 (1999).
8. Akira, S., Takeda, K. & Kaisho, T. Toll-like receptors: critical proteins linking innate and acquired immunity. *Nat. Immunol.* **2**, 675–680 (2001).
9. Benigni, A. *et al.* Involvement of renal tubular Toll-like receptor 9 in the development of tubulointerstitial injury in systemic lupus. *Arthritis Rheum.* **56**, 1569–1578 (2007).
10. Wu, X. & Peng, S. L. Toll-like receptor 9 signaling protects against murine lupus. *Arthritis Rheum.* **54**, 336–342 (2006).
11. Stoehr, A. D. *et al.* TLR9 in peritoneal B-1b cells is essential for production of protective self-reactive IgM to control T_H17 cells and severe autoimmunity. *J. Immunol.* **187**, 2953–2965 (2011).
12. Ishii, K. J. *et al.* A Toll-like receptor-independent antiviral response induced by double-stranded B-form DNA. *Nat. Immunol.* **7**, 40–48 (2006).
13. Ishikawa, H., Ma, Z. & Barber, G. N. STING regulates intracellular DNA-mediated, type I interferon-dependent innate immunity. *Nature* **461**, 788–792 (2009).
14. Takaoka, A. *et al.* DAI (DLM-1/ZBP1) is a cytosolic DNA sensor and an activator of innate immune response. *Nature* **448**, 501–505 (2007).
15. Ablasser, A. *et al.* RIG-I-dependent sensing of poly(dA:dT) through the induction of an RNA polymerase III-transcribed RNA intermediate. *Nat. Immunol.* **10**, 1065–1072 (2009).
16. Muruve, D. A. *et al.* The inflammasome recognizes cytosolic microbial and host DNA and triggers an innate immune response. *Nature* **452**, 103–107 (2008).

17. Roberts, T. L. *et al.* HIN-200 proteins regulate caspase activation in response to foreign cytoplasmic DNA. *Science* **323**, 1057–1060 (2009).
18. Decker, P., Singh-Jasuja, H., Haager, S., Kötter, I. & Rammensee, H. G. Nucleosome, the main autoantigen in systemic lupus erythematosus, induces direct dendritic cell activation via a MyD88-independent pathway: consequences on inflammation. *J. Immunol.* **174**, 3326–3334 (2005).
19. Hägele, H. *et al.* Double-stranded DNA activates glomerular endothelial cells and enhances albumin permeability via a Toll-like receptor-independent cytosolic DNA recognition pathway. *Am. J. Pathol.* **175**, 1896–1904 (2009).
20. Allam, R. *et al.* Viral RNA and DNA trigger common antiviral responses in mesangial cells. *J. Am. Soc. Nephrol.* **20**, 1986–1996 (2009).
21. Leadbetter, E. A. *et al.* Chromatin-IgG complexes activate B cells by dual engagement of IgM and Toll-like receptors. *Nature* **416**, 603–607 (2002).
22. Means, T. K. *et al.* Human lupus autoantibody-DNA complexes activate DCs through cooperation of CD32 and TLR9. *J. Clin. Invest.* **115**, 407–417 (2005).
23. Lande, R. *et al.* Plasmacytoid dendritic cells sense self-DNA coupled with antimicrobial peptide. *Nature* **449**, 564–569 (2007).
24. Tian, J. *et al.* Toll-like receptor 9-dependent activation by DNA-containing immune complexes is mediated by HMGB1 and RAGE. *Nat. Immunol.* **8**, 487–496 (2007).
25. Yanai, H. *et al.* HMGB proteins function as universal sentinels for nucleic-acid-mediated innate immune responses. *Nature* **462**, 99–103 (2009).
26. van Bruggen, M. C. *et al.* Heparin and heparinoids prevent the binding of immune complexes containing nucleosomal antigens to the GBM and delay nephritis in MRL/lpr mice. *Kidney Int.* **50**, 1555–1564 (1996).
27. Hedberg, A. *et al.* Heparin exerts a dual effect on murine lupus nephritis by enhancing enzymatic chromatin degradation and preventing chromatin binding in glomerular membranes. *Arthritis Rheum.* **63**, 1065–1075 (2011).
28. Napirei, M., Ludwig, S., Mezrhab, J., Klöckl, T. & Mannherz, H. G. Murine serum nucleases —contrasting effects of plasmin and heparin on the activities of DNase1 and DNase1-like 3 (DNase1l3). *FEBS J.* **276**, 1059–1073 (2009).
29. Reilly, C. M. *et al.* The histone deacetylase inhibitor trichostatin A upregulates regulatory T cells and modulates autoimmunity in NZB/W F1 mice. *J. Autoimmun.* **31**, 123–130 (2008).
30. Hsiang, Y. H., Lihou, M. G. & Liu, L. F. Arrest of replication forks by drug-stabilized topoisomerase I–DNA cleavable complexes as a mechanism of cell killing by camptothecin. *Cancer Res.* **49**, 5077–5082 (1989).
31. Hsiang, Y. H., Hertzberg, R., Hecht, S. & Liu, L. F. Camptothecin induces protein-linked DNA breaks via mammalian DNA topoisomerase I. *J. Biol. Chem.* **260**, 14873–14878 (1985).
32. Frese-Schaper, M., Zbaeren, J., Gugger, M., Monestier, M. & Frese, S. Reversal of established lupus nephritis and prolonged survival of New Zealand black × New Zealand white mice treated with the topoisomerase I inhibitor irinotecan. *J. Immunol.* **184**, 2175–2182 (2010).
33. Samaha, R. J. & Irvin, W. S. Deoxyribonucleic acid strandedness. Partial characterization of the antigenic regions binding antibodies in lupus erythematosus serum. *J. Clin. Invest.* **56**, 446–457 (1975).
34. Samejima, K. & Earnshaw, W. C. Trashing the genome: the role of nucleases during apoptosis. *Nat. Rev. Mol. Cell Biol.* **6**, 677–688 (2005).
35. Campbell, V. W. & Jackson, D. A. The effect of divalent cations on the mode of action of DNase I. The initial reaction products produced from covalently closed circular DNA. *J. Biol. Chem.* **255**, 3726–3735 (1980).
36. Chitrabamrung, S., Rubin, R. L. & Tan, E. M. Serum deoxyribonuclease I and clinical activity in systemic lupus erythematosus. *Rheumatol. Int.* **1**, 55–60 (1981).
37. Fenton, K. *et al.* Anti-dsDNA antibodies promote initiation, and acquired loss of renal Dnase1 promotes progression of lupus nephritis in autoimmune (NZB×NZW)F1 mice. *PLoS ONE* **4**, e8474 (2009).
38. Macanovic, M. *et al.* The treatment of systemic lupus erythematosus (SLE) in NZB/W F1 hybrid mice; studies with recombinant murine DNase and with dexamethasone. *Clin. Exp. Immunol.* **106**, 243–252 (1996).
39. Verthelyi, D., Dybdal, N., Elias, K. A. & Klinman, D. M. DNAse treatment does not improve the survival of lupus prone (NZB × NZW)F1 mice. *Lupus* **7**, 223–230 (1998).
40. Venkatesh, J. *et al.* Antigen is required for maturation and activation of pathogenic anti-DNA antibodies and systemic inflammation. *J. Immunol.* **186**, 5304–5312 (2011).
41. Neubert, K. *et al.* The proteasome inhibitor bortezomib depletes plasma cells and protects mice with lupus-like disease from nephritis. *Nat. Med.* **14**, 748–755 (2008).

Author contributions

S. Frese researched the data for the article and wrote the article. B. Diamond contributed to review and/or editing of the manuscript before submission. S. Frese and B. Diamond contributed equally to discussions of the content.