Involvement of high mobility group box 1 in the auto-inflammatory process in systemic lupus erythematosus
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CHAPTER 8
Summary, discussion and future perspectives
In this thesis we investigated the role of High Mobility Group Box 1 (HMGB1), in the auto-inflammatory process in Systemic Lupus Erythematosus (SLE). An overview of the current literature on the role of HMGB1 in SLE is given in Chapter 2. More specifically, we studied the effects of HMGB1 on macrophage polarization, phagocytosis and cytokine production (Chapter 3). Furthermore, we evaluated whether auto-antibodies directed against HMGB1 could be of clinical relevance in SLE patients (Chapter 4) and evaluated whether HMGB1 is a potential therapeutic target in SLE (Chapter 6). Per chapter the results are discussed, limitations are described and future perspectives are given.

Chapter 2: SLE and HMGB1: An Overview

SLE is a systemic autoimmune disease, characterized by hyperactivation of immune cells, production of anti-nuclear auto-antibodies, and multiple organ damage triggered by immune complex deposition (1,2). It is assumed that a disturbed clearance of apoptotic cells plays a central role in the pathogenesis (3). When apoptotic cells are not cleared, these cells will turn into necrotic cells which lose their membrane integrity thereby releasing intracellular structures, which contain modified proteins that can act as autoantigens. These structures can be captured by antigen presenting cells, and through interactions with T- and B cells lead to auto-antibody production. These auto-antibodies can cause formation of circulating immune complexes that deposit in organs, such as the kidney and skin resulting in inflammation and tissue damage (4).

HMGB1 and other damage associated molecular pattern (DAMP) molecules can be released due to disturbed clearance of apoptotic cells or tissue damage. HMGB1 can be released from activated, apoptotic and necrotic cells. When released, HMGB1 can recruit immune cells to the site of inflammation and activate these cells to secrete pro-inflammatory cytokines (5). HMGB1 can induce cytokine production via two different pathways. First, HMGB1 can engage and activate DAMP receptors including TLR2, TLR4, TLR9 and the Receptor for Advanced Glycation Endproducts (RAGE), depending on its post-translational modifications such as acetylation, phosphorylation, methylation and redox changes of cysteine residues. Second, HMGB1 can form complexes with other molecules, for instance lipopolysaccharides (LPS) or interleukin (IL)-1, which will subsequently amplify the pro-inflammatory effects of these molecules when they bind to their receptors. These findings suggest that HMGB1 plays a pivotal role in initiating inflammation in response to tissue damage. Compared to healthy controls (HC), levels of serum HMGB1 are increased in SLE patients (6-8). Moreover, increased extracellular levels of HMGB1 have been found locally in the skin (9-11) and kidneys (12,13),
organs typically effected by the disease process. Finally, increased levels of auto-
antibodies to HMGB1 have been detected in sera of SLE patients compared to
healthy individuals but the clinical relevance of these auto-antibodies is still unclear
(6,14,15). Taken together, the available data suggest that HMGB1 production and/
or its release is increased in SLE, which will lead to an excessive inflammatory
response and progressive tissue damage. Thus, HMGB1 could be a potential target
for intervention.

CHAPTER 3: HMGB1 AND ITS EFFECT ON MACROPHAGE
POLARIZATION AND PHAGOCYTOSIS

In this chapter we investigated whether increased HMGB1 levels result in a more
pro-inflammatory phenotype of macrophages. As there is impaired clearance and
accumulation of apoptotic debris in SLE this can lead to increased HMGB1 levels
locally. Moreover, it has been reported that HMGB1 can contribute to a decrease in
phagocytic capacity of macrophages by masking phosphatidylserine on apoptotic
cells (16), a strong eat-me signal for macrophages. Thus, in SLE a vicious circle might
be operant that is initiated by tissue damage resulting in formation of apoptotic
debris that will stimulate HMGB1 secretion which in turn will hamper, by altering
phagocytic capacity of macrophages, the clearance of the apoptotic material. Also, increased HMGB1 levels might skew differentiation of monocytes into M1
macrophages, instead of M2 macrophages and as M1 macrophages are less
efficient phagocytes than M2 macrophages this might further fuel the inflammatory
response in SLE.

Therefore, the effect of HMGB1 on macrophage polarization and on phagocytic
capacity of differentiated macrophages was investigated. Our results indicate
that circulating monocytes from SLE patients display a more M1-like phenotype
compared to HC, but in vitro differentiation abolishes this difference. In vitro,
HMGB1 was able to skew the phenotype of M2-like (M-CSF) differentiated
macrophages towards a pro-inflammatory M1-like macrophage as evidenced by
increased mRNA levels of IL-6 and TNF-α. Addition of HMGB1 during differentiation
led to M1-like macrophages with reduced phagocytic capacity. Our data also
indicate that HMGB1 can contribute directly to the impaired phagocytic capacity
as seen in SLE patients by interfering with recognition of apoptotic cells, as pre-
incubation of apoptotic cells with HMGB1 resulted in reduced phagocytosis. These
data suggest that HMGB1, when present locally, may play a role in sustaining the
inflammatory loop through either increasing the apoptotic load (by a decreased
phagocytosis) or by skewing the phenotype of M2-like macrophage towards a more
pro-inflammatory M1-like macrophage.
Limitations

It should be noted that the redox status of the HMGB1 preparation used in our study is unknown. Recent studies have indicated that post-translational modifications to HMGB1 can alter its function and localization. For example, several studies have demonstrated that when a disulfide bridge exists between cysteines C23 and C45, and cysteine C106, HMGB1 is in a thiol (reduced) state and exerts cytokine inducing properties via binding to TLR4 (17-19). As shown in chapter 3 the commercially available HMGB1 preparation used in our experiments was able to induce cytokine production from macrophages, indicating that the HMGB1 employed in these experiments was most probably in the cytokine inducing form. Two other forms of HMGB1 exist, one which has chemoattractant properties when all three cysteines are reduced, and one which is immunologically silent (19,20). This immunologically silent form of HMGB1 can be released from apoptotic cells. It has no chemotactic properties, as its three cysteines are terminally oxidized by reactive oxygen species (ROS) to sulfonates (18,19,21). However, when necrotic cells release HMGB1, it still exerts pro-inflammatory properties (22) and can form complexes with nucleosomes (23) or DNA (24,25). In the context of SLE, these findings are important as aberrant phagocytosis leads to formation of secondary necrotic cells which can consequently lead to HMGB1 containing immune complexes or HMGB1 initiated inflammation.

Future perspectives

Until now only one study reported the redox status of HMGB1 in serum of SLE patients. In this study it was demonstrated that in four patients with active disease the oxidized form of HMGB1 was present (26). As this was only a small study, further research is needed to determine the redox status of HMGB1 in SLE patients. Particularly, it will be interesting to investigate whether there are differences in HMGB1 redox status between active and quiescent SLE patients, as this may give further insights into the role of HMGB1 in the pathogenesis of SLE. Furthermore, the kinetics of HMGB1 isoforms could be studied in a longitudinal study since HMGB1 isoforms might change over time from chemoattractant to cytokine-inducing forms during or before an exacerbation. In four patients with the macrophage activation syndrome (MAS) different isoforms of HMGB1 were measured longitudinally by tandem mass spectrometry, before and after treatment (27). This study, albeit in a small number of patients, demonstrated that during active disease cytokine-inducing HMGB1 was present while after treatment with etoposide, a topoisomerase inhibitor that boosts apoptotic cell death, the oxidized non-inflammatory isoform emerged (27). Studying different isoforms of HMGB1 can help to elucidate the source of HMGB1 in SLE. For example, it is unclear whether HMGB1 in the kidneys, is derived from apoptotic cells or activated renal...
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and inflammatory cells or both. As the redox status of HMGB1 released from apoptotic cells is different from HMGB1 secreted from activated cells (19), tandem mass spectrometry could be used to investigate these differences.

Another mechanism by which locally released HMGB1 can contribute to inflammation is by inducing cell death directly. In cancer research it has been demonstrated that excessive extracellular accumulation of HMGB1 is toxic to cells and causes cell death (28). Furthermore, tumor cells triggered by pro-apoptotic agents are sensitive to HMGB1-induced cell death characterized by the formation of giant mitochondria (29,30) although the exact mechanisms underlying the increased sensitivity of tumor cells to HMGB1 induced cell death are currently unknown. However, these studies indicate that under specific circumstances, HMGB1 may increase cell death, which might lead to further accumulation of necrotic material in SLE although so far this has not been studied.

Chapter 4: Auto-antibodies to HMGB1 as a Marker of Disease?

As mentioned above, HMGB1 is considered an important pro-inflammatory mediator in the pathogenesis of SLE and a potential biomarker of disease activity. Interestingly, a number of studies have also demonstrated the occurrence of auto-antibodies against HMGB1 in SLE (6,14,15,31). In a previous study from our group, a relation was found with disease activity as anti-HMGB1 levels, albeit weakly, correlated with the SLE disease activity index (SLEDAI) and anti-dsDNA levels (6) which was confirmed in a recent Swedish study (31). In this chapter we investigated the clinical relevance of auto-antibodies against HMGB1 in SLE further as well as the specific reactivity to Box A and Box B, which are the major domains of the HMGB1 molecule. Anti-HMGB1 levels were measured in a newly established patient cohort and, in line with previous studies, we found that anti-HMGB1 antibody levels in SLE were increased compared to HC. However, no correlation was found between variables of disease activity and IgG anti-HMGB1 levels. Also, associations with organ involvement, as defined by ACR criteria, were inconsistent. Patients with neurological disorders had lower levels of anti-HMGB1 whereas patients with arthritis had higher levels of anti-HMGB1 but no relation with disease activity, serum complement C3 levels or anti-dsDNA antibodies was observed.

The reasons for these apparent discrepancies between the present and previous studies are unclear but may be explained by differences in the SLE patient cohorts studied and the assay system used to detect anti-HMGB1 antibodies. In the anti-HMGB1 assay presented in this chapter care was taken to correct for unspecific binding and data were expressed as arbitrary units against a reference serum sample from a SLE patient with high anti-HMGB1 IgG levels.
In conclusion, based on the data presented in this chapter, we confirm that levels of anti-HMGB1 are elevated in SLE but measuring these levels does not constitute a reliable indicator for disease activity. In contrast to total anti-HMGB1 levels, antibody levels specifically directed to the Box A domain did show a positive correlation with disease activity and demonstrated a high sensitivity for SLE. Therefore, anti-Box A antibody levels might be a better biomarker compared to total anti-HMGB1. However, these results clearly need to be confirmed in larger and, preferably, independent cohorts of SLE patients which also should include disease controls to determine whether anti-Box A antibodies are specific for SLE. Additionally, longitudinal studies are needed to investigate whether anti-Box A levels might be a novel marker for active disease in individual patients.

**Limitations**

In our study we did not investigate whether anti-HMGB1-HMGB1 complexes were present in the serum of SLE patients. Potentially, such complexes can interfere with the detection of anti-HMGB1 antibodies. To explore this hypothesis, a competition ELISA system could be used in which increasing concentrations of recombinant HMGB1 are added to serum in which high levels of anti-HMGB1 antibodies are present.

**Future perspectives**

As mentioned previously, in SLE patients circulating HMGB1 levels are also increased. At present, it is not clear whether an increase in HMGB1 levels precedes an increase in anti-HMGB1 levels or whether these changes occur in parallel. Theoretically, increased levels of the autoantigen HMGB1 could stimulate the formation of anti-HMGB1 antibodies. To further investigate this, longitudinal studies in which HMGB1 and anti-HMGB1 are measured concurrently in the same patient could performed. This may give insight into whether HMGB1 and anti-HMGB1 levels change over time in a similar pattern.

As we observed a relation between disease activity and anti-Box A antibodies, further investigations should focus on why specifically anti-Box A levels are increased during active disease and whether this might interfere with the cytokine inducing properties of HMGB1. In this respect, more detailed mapping of the epitopes recognized by the anti-HMGB1 autoantibodies may be helpful to reveal whether epitope specificities exist that potentially impact the disease course. Until now only one study has investigated anti-HMGB1 autoantibody epitopes in SLE showing that in SLE patients antibodies are predominantly directed against Box A (15).
CHAPTER 5: HMGB1 AS A TARGET FOR INTERVENTION, A REVIEW

Although the exact role of endogenously formed anti-HMGB1 antibodies in the pathogenesis of SLE still needs more clarification, data from animal studies indicate that treatment with monoclonal or polyclonal anti-HMGB1 antibodies can ameliorate disease development in various models of inflammation and autoimmunity (32-35).

In a broader perspective, it is interesting to note that there are multiple ways to achieve inhibition of HMGB1. One example is glycyrrhizin, a traditional herbal medicine which can bind directly to HMGB1 and thereby inhibit its chemoattractant activities (36). Treatment with glycyrrhizin has been shown to alleviate lupus nephritis in an activated lymphocyte derived (ALD)-DNA induced lupus mice model (37). However, a range of biological effects have been reported for glycyrrhizin, including anti-inflammatory, anti-ulcerative, cytoprotective and cytotoxic activities (36,38). Therefore, it is unlikely that glycyrrhizin exerts its beneficial effects solely via blocking of HMGB1. Another approach to block HMGB1 mediated effects is using recombinant Box A, which acts as a competitive antagonist for HMGB1 binding to its receptor RAGE. Treatment with Box A has shown beneficial effects in mouse models of sepsis (39) and arthritis (32,40). Soluble RAGE (sRAGE) is another therapeutic option which can be explored since it has been demonstrated that sRAGE can bind uncomplexed HMGB1 in serum (41). Furthermore, it has been shown that sRAGE levels are decreased in SLE compared to HC (42-44). Indeed, treatment with sRAGE significantly improved proteinuria and renal damage, reduced glomerular immune complex deposition and serum concentrations of lupus nephritis–related auto-antibodies in NZBxNZW mice, a mouse strain that spontaneously develops a lupus like syndrome (45). However, in this study it is unclear whether treatment with sRAGE did indeed bind HMGB1, as HMGB1 levels were not measured. Moreover, RAGE has several other ligands, and it is therefore unclear whether the positive effects demonstrated in this study can be solely attributed to blocking HMGB1. Furthermore, deletion of RAGE in B6/lpr mice exacerbated lupus nephritis and the autoimmune lymphoproliferative syndrome (46), clearly indicating that more studies are required to evaluate whether blocking RAGE is a safe option in SLE patients. Finally, HMGB1 can be targeted by drugs which inhibit its translocation from the nucleus. These include for instance metformin, a known anti-diabetic drug (47), and compounds that inhibit the Calcium/calmodulin-dependent protein kinase (CaMK) IV pathway (48). Metformin has demonstrated promising effects in two mouse models of SLE, where it normalized T cell metabolism (49) and proved beneficial as an add-on therapy in SLE patients (50). Inhibition of CAMK IV reduced mortality in MRL/lpr mice and limited production of IL-17 (51). However, the role of HMGB1 was not investigated directly in these studies and it is unknown...
whether inhibition of translocation of HMGB1 from the nucleus contributed to the positive effects observed. It must be noted that HMGB1 has cytosolic functions, thus by inhibiting translocation not only extracellular release of HMGB1 will be affected. It has recently been discovered that HMGB1 plays an important role in the promotion of autophagy (52), a conserved programmed survival pathway evoked following environmental and intracellular stress, while inhibiting apoptosis (52,53). Several lines of evidence point towards an increase in autophagy in SLE as well (54-58). Given the link between cytosolic HMGB1 and increased autophagy (52,53), this suggests that retention of HMGB1 in the nucleus might be beneficial in SLE. However, the absence of cytosolic HMGB1 has also been associated with an increase in apoptosis (52,53), and as the load of apoptotic debris is already elevated in SLE caution should be taken to deplete HMGB1 in the cytosol.

CHAPTER 6: NEUTRALIZING HMGB1 IN MRL/LPR MICE

Although there is indirect evidence that inhibition of HMGB1 might be beneficial in SLE, only few studies have directly addressed this topic. Therefore, in this chapter, we tested the hypothesis that lupus nephritis and disease progression in lupus prone mice is ameliorated by anti-HMGB1 monoclonal antibodies that neutralize HMGB1. In this study, MRL/lpr mice, which develop a SLE-like, systemic autoimmune disease with lymphadenopathy, auto-antibody production, complement activation and glomerulonephritis, were treated with monoclonal anti-HMGB1 (2G7) or isotype control antibodies. The anti-HMGB1 monoclonal antibody 2G7 has been extensively characterized previously with respect to its HMGB1 neutralizing activity in cell culture assays, of both human and mouse macrophages, and in animal models of HMGB1-mediated damage, such as sepsis (17,39,59-63). Moreover, the 2G7 anti-HMGB1 mAb has been shown to specifically neutralize the cytokine isoform of HMGB1 (17). Contrary to our expectations, intraperitoneal administration of anti-HMGB1 2G7 to lupus-prone MRL/lpr mice (50 µg/mouse twice weekly during 10 weeks) did not ameliorate glomerulonephritis. Also, no differences were observed with respect to circulating levels of complement C3, anti-dsDNA antibodies and pro-inflammatory cytokines between mice treated with control antibody or mice treated with mAb 2G7 at any time point. Interestingly, it has been reported that MRL/lpr mice deficient for either TLR2 or TLR4 develop glomerulonephritis to a similar extent as wildtype MRL/lpr mice (64). As both TLR2 and TLR4 are known receptors for HMGB1, this could potentially explain our negative findings. However, as HMGB1 can also signal through TRL9 or RAGE, this only indicates that HMGB1 – TLR2/4 signaling might not be important in disease development in MRL/lpr mice. Clearly, further studies are needed to validate our results in other mouse models of SLE.
**Limitations**

One explanation for these negative results might be that in this chronic mouse model disease develops gradually and that HMGB1 most likely represents only one of many pro-inflammatory factors which can activate the immune system and cause damage. Another explanation might be that with systemic administration of an antibody only circulating HMGB1 is targeted. HMGB1 staining in the kidneys of our mice indicated local release of HMGB1 as extracellular presence of HMGB1 was observed. Furthermore, activated lymphocytes can secrete HMGB1, and high levels of HMGB1 can be present locally in spleen and lymph nodes. This locally released HMGB1 might not be efficiently targeted by the intraperitoneally administered monoclonal anti-HMGB1 antibodies although previous studies in BXSB lupus mice and a sepsis model have clearly shown a beneficial effect of anti-HMGB1 antibody treatment despite development of splenomegaly (60, 65).

Although MRL/lpr mice are considered a robust model for lupus nephritis, the pathogenic process is largely dependent on the accumulation of autoreactive CD4 T cells and B cells due to the defect in apoptosis (66). As shown in chapter 3, HMGB1 plays a role in the pathogenesis of SLE by decreasing phagocytosis and therefore it might be an interesting option to choose a mouse model in which altered phagocytosis is known to play a role. For example, mice lacking C1q or Milk fat globule-EGF factor 8 protein (MFGE8) develop lupus like syndromes due to defects in phagocytosis, which might resemble the pathogenic process of SLE more accurately (67).

**Future perspectives**

Another approach to investigate the role of HMGB1 in SLE is to use mouse models with tissue or cell specific ablation of HMGB1. Full HMGB1 knock-out mice die within 24 hours after birth due to severe hypoglycemia, but fibroblast cell lines derived from these mice can proliferate and survive indicating that these effects might be cell specific (68). Recently, \textit{Hmgb1}-floxed (\textit{Hmgb1}f/f) mice have been generated that enable the cell- and tissue-specific deletion of the gene when crossed with an appropriate \textit{Cre} recombinase transgenic strain (53, 69, 70). As HMGB1 is an important mediator for many different immune cells (71), it might be worthwhile to investigate whether mice which are deficient for HMGB1 in the hematopoietic compartment are protected against SLE. It could also be interesting to generate mice in which specific immune cells, such as macrophages, are made deficient for HMGB1. These mice can then be backcrossed with mouse strains that spontaneously develop chronic SLE or be used in models in which lupus nephritis is induced by for instance injection of pristine (66) or ALD-DNA (72).
CHAPTER 7: HMGB1 AND IL-17

Studies have indicated that IL-17 plays a role in SLE pathogenesis and blocking IL-17A exerted positive effects in mouse models of SLE (73-76). Although a role for IL-17A in SLE has been suggested, the role of other IL-17A family members in SLE has not been directly studied in mice. Since the IL-17RA subunit is a common receptor unit for most if not all IL-17 family members (77), this chapter investigated the role of IL-17 receptor A (RA) signaling in the pathogenesis of SLE. Recently, several studies reported possible links between IL-17 and HMGB1 (78-80) and it has been demonstrated that blocking HMGB1 in animal models leads to a decrease in IL-17 levels in serum and bronchoalveolar lavage fluid (81,82). It is thought that HMGB1 can induce production of IL-17 through TLR2-IL-23 (79,83) or TLR4-IL-6 (80) interactions. We crossed B6/lpr mice with IL17RA KO mice to investigate the role of IL17RA signaling in SLE. The results show that IL-17 signaling might limit lymphoproliferation as IL17RA KO/lpr mice showed increased lymphadenopathy and splenomegaly. In contrast, there was no change in ANA titers and in both IL17RA KO/lpr and B6/lpr mice no development of glomeronephritis was seen. A significant increase in HMGB1 and anti-HMGB1 antibodies was noted before clinical signs of lymphoproliferative disease were observed. One explanation for the increased HMGB1 levels might be that these reflect the lymphadenopathy/splenomegaly seen in IL17RA KO/lpr mice as activated lymphoid cells can actively secrete HMGB1 (71).

At 30 weeks of age the IL17RA KO/lpr mice displayed high levels of anti-HMGB1 antibodies, which were comparable to levels observed in untreated MRL/lpr mice at 17 weeks as described in chapter 6. Interestingly, the increased levels of anti-HMGB1 antibodies did not seem to have an effect on SLE features in IL17RA KO/lpr, although it should be noted that the SLE phenotype is milder in B6/lpr mice compared to MRL/lpr mice (66,84) making it difficult to draw direct parallels between the studies. Also, in chapter 4, no correlations between circulating levels of anti-HMGB1 antibodies and clinical parameters in SLE patients were found.

Limitations

Noticeably, the high levels of HMGB1 detected in the IL17RA KO/lpr and B6/lpr mice were not related to ANA titers. Previous studies have suggested that HMGB1, present in immune complexes, can contribute to ANA, and specifically anti-dsDNA, formation (24,85). It has been demonstrated that the presence of HMGB1 in these complexes is required for induction of anti-dsDNA antibodies (24). As we did not measure immune complex formation and the presence of HMGB1 therein, it is difficult to draw firm conclusions at this stage. However, it can be concluded that an increase in HMGB1 per se does not directly lead to an increase in ANA, indicating that other factors are needed as well.
**Future perspectives**

To investigate whether the lymphadenopathy and splenomegaly in IL17RA KO/lpr is due to an increase in lymphoproliferation or decrease in apoptosis further studies are needed. For instance, T cells from the lymph nodes of IL17RA KO/lpr mice and B6/lpr mice could be isolated and stimulated ex vivo with anti-CD3 and anti-CD28. Afterwards, cells can be labeled with carboxyfluorescein succinimidyl ester (CFSE) to measure proliferation, or Annexin 5 and propidium iodide (PI) staining can be used to measure apoptosis. To investigate whether HMGB1 contributes to increased lympoproliferation, recombinant HMGB1 could be added to these cultures.

Furthermore, more studies are required to investigate the mechanism behind the increased HMGB1 levels observed and the possible existence of a negative feedback loop between IL-17RA signaling and HMGB1. As has been proposed in the literature, HMGB1 release is most likely upstream from IL-17 signaling (78-80). Therefore, it might be interesting to block HMGB1 in (young) IL17RA KO/lpr mice to investigate effects on IL-17 levels in the absence of IL-17 RA signaling. To further investigate whether IL-17RA signaling itself has an effect on HMGB1 levels, IL-17 can be added or blocked in cell cultures from lymph nodes of B6/lpr mice. Although such experiments may not give direct evidence towards the existence of a negative feedback loop they may provide first clues.

**CONCLUDING REMARKS**

In summary, the results in this thesis provide further evidence that HMGB1 is involved in SLE pathogenesis. In particular, it was demonstrated that HMGB1 contributes to the impaired phagocytosis capacity of macrophages, as seen in SLE, by interfering with recognition of apoptotic cells as well as by influencing the macrophage itself. As such, HMGB1 can be considered an important factor in sustaining the auto-inflammatory processes in SLE. However, treatment with a neutralizing monoclonal anti-HMGB1 antibody does not inhibit development or affect progression of lupus nephritis in MRL/lpr mice but further studies are warranted in other mouse models of SLE to validate these results. Finally, in SLE patients, auto-antibodies against total HMGB1 do not seem a useful indicator for disease activity. These observations suggest that anti-HMGB1 antibodies do not directly affect the disease process but develop as a bystander effect of the immunological aberrations and severe inflammation seen in SLE. However, antibodies directed against the Box A domain of HMGB1 might be a promising new biomarker. Further research should focus on delineating the kinetics, cellular location and redox status of HMGB1 in SLE and a more detailed analysis of the epitope specificities of the anti-HMGB1 autoantibodies.
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