Involvement of high mobility group box 1 in the auto-inflammatory process in systemic lupus erythematosus
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CHAPTER 1

Brief introduction and aim of the thesis
Systemic Lupus Erythematosus (SLE) is a systemic autoimmune disease of unknown cause which affects mainly women of childbearing age, with a female to male ratio of 9:1 (1). The disease is characterized by a wide array of clinical manifestations ranging from butterfly rash and arthritis to renal involvement (2) as reflected by the diagnostic criteria of American College of Rheumatology (ACR) (3) (table 1). Patients are diagnosed with SLE when at least four of these criteria are present. Glomerulonephritis leading to persistent proteinuria and chronic renal failure is one of the most severe complications and is associated with significant mortality (1).

Table 1: American College of Rheumatology (ACR) criteria for diagnosis of SLE.

<table>
<thead>
<tr>
<th>Criterion</th>
<th>Definition</th>
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<tbody>
<tr>
<td>Malar rash</td>
<td>butterfly-shaped rash across cheeks and nose</td>
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<tr>
<td>Discoid rash</td>
<td>raised red patches</td>
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<tr>
<td>Photosensitivity</td>
<td>skin rash as result of unusual reaction to sunlight</td>
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<tr>
<td>Oral ulcers</td>
<td>oral or nasopharyngeal ulceration</td>
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<tr>
<td>Arthritis</td>
<td>in two or more joints, along with tenderness, swelling, or effusion</td>
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<tr>
<td>Serositis</td>
<td>inflammation of the lining around the heart (pericarditis) and/or lungs (pleuritis)</td>
</tr>
<tr>
<td>Renal disorder</td>
<td>excessive protein in the urine, or cellular casts in the urine</td>
</tr>
<tr>
<td>Neurological disorder</td>
<td>seizures and/or psychosis</td>
</tr>
<tr>
<td>Hematological disorder</td>
<td>hemolytic anemia, low white blood cell count, or low platelet count</td>
</tr>
<tr>
<td>Immunological disorder</td>
<td>antibodies to double stranded DNA, antibodies to Sm, or antibodies to cardiolipin</td>
</tr>
<tr>
<td>Presence of anti-nuclear antibodies</td>
<td>a positive test in the absence of drugs known to induce it</td>
</tr>
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</table>

Genetic factors confer a predisposition to SLE, as genome wide association studies (GWAS) have found associations with several single nucleotide polymorphisms (SNPs) (1). For instance, variation in the ITGAM gene is one the strongest risk factors for SLE (4). ITGAM encodes for CD11-b, which is an important receptor for phagocytosis, and SNPs in ITGAM can lead to impaired phagocytosis (4). Environmental factors, such as sunlight exposure or smoking, can also contribute to the development of the disease (1,5).

Furthermore, SLE is strongly associated with defects in clearance of apoptotic cells which is thought to be due to aberrant phagocytosis. Under homeostatic circumstances the detection and removal of apoptotic cells generally occurs rapidly and induces anti-inflammatory responses (6). Dying cells are removed by professional phagocytes, such as macrophages or dendritic cells (DC’s). In patients
with SLE, there is accumulation of apoptotic cells in the skin following exposure to ultraviolet radiation and an increase in spontaneous appearance of apoptotic cells in blood and lymph nodes (7,8). Evidence from animal models further substantiates these findings, as mice deficient in complement components, which are important for efficient phagocytosis, develop lupus-like disease (9). Moreover, in serum from SLE patients lower levels of complement factors are associated with a decrease in phagocytosis (10). Additionally, auto-antibodies against complement have been found in patients with active lupus nephritis (11) and in vitro these antibodies interfered with the phagocytosis of C1q opsonized apoptotic cells by macrophages (12). The resulting increase in apoptotic cells and delayed phagocytosis eventually leads to secondary necrosis and allows the release of intracellular contents. This leads to increased exposure of self-antigens to the adaptive immune system. This, in turn, can result in the generation of auto-antibodies, especially auto-antibodies directed against nuclear structures such as anti-double stranded DNA antibodies, which are a hallmark of the disease. The presence of these auto-antibodies results in immune complex formation that deposit in organs, such as the kidney. These immune complexes can activate innate immune cells by binding to Fc receptors and can initiate complement system activation. Consequently this leads to the release of toxic products, such as oxygen radicals and proteolytic enzymes, and the production of pro-inflammatory cytokines and chemokines resulting in progressive inflammation and eventually tissue damage (5).

HIGH MOBILITY GROUP BOX 1 AND SLE

High Mobility Group Box 1 (HMGB1) is a nuclear protein, which is ubiquitously expressed. Depending on its location and posttranslational modifications it can serve different functions. As a nuclear protein, HMGB1 stabilizes the nucleosomal structure enables the binding of transcription factors to their cognate DNA sequences, and facilitates gene transcription (13). HMGB1 can be released from the cell into the extracellular space where it acts as a damage-associated molecular pattern (DAMP) molecule or alarmin. Secretion can occur actively from macrophages and other immune cells upon stimulation by pro-inflammatory factors or passively during apoptosis and necrosis. When present in the extracellular space, HMGB1 can bind to several receptors including the Receptor for Advanced Glycation End products (RAGE) and Toll Like Receptor 4 (TLR4) (14) and can promote several pro-inflammatory responses. For example, it stimulates the release of inflammatory cytokines like tumor necrosis factor (TNF-α) and interleukin (IL)-6 from monocytes/macrophages (15-18). In addition, HMGB1 has been demonstrated to promote cell
Brief introduction and aim of the thesis

In the context of SLE, it has been shown that nucleosomes, bound to the nuclear protein HMGB1, induce not only immune but also inflammatory responses (22). Furthermore, several studies have demonstrated that, compared to healthy individuals, serum and urinary levels of HMGB1 are increased in SLE and correlate with disease activity (23-27). Finally, the occurrence of auto-antibodies against HMGB1 in SLE has been described (28-31), although the clinical relevance of these auto-antibodies is unclear. Collectively, these studies suggest that HMGB1 is an important factor in the pathogenesis of SLE and could constitute a potential therapeutic target. However, the underlying mechanisms by which HMGB1 contributes to SLE pathogenesis are not fully understood.

AIM OF THIS THESIS

The research presented in this thesis centers around the role of HMGB1 in the auto-inflammatory process in SLE. More specifically, within this thesis, the effects of HMGB1 on macrophage polarization, phagocytosis and cytokine production were studied. Furthermore, we evaluated whether HMGB1 is a potential therapeutic target in SLE and investigated whether auto-antibodies directed against HMGB1 could be of clinical relevance in SLE patients.

In chapter 2 the literature on HMGB1, and its (potential) contribution to the pathogenesis of SLE is discussed. We hypothesized that increased HMGB1 levels, as seen in SLE, might lead to skewing of monocyte differentiation towards pro-inflammatory M1 macrophages, instead of anti-inflammatory M2 macrophages. M1 macrophages are implicated in initiating and sustaining inflammation while M2 macrophages are associated with resolution or smouldering chronic inflammation (32). As M1 macrophages are less efficient phagocytes this might fuel the inflammatory response in SLE. Therefore, in chapter 3 monocytes and macrophages of SLE patients were investigated regarding the expression of M1 and M2 specific surface markers and phagocytosis capacity. In addition, the direct effect of HMGB1 on macrophage polarization and phagocytosis capacity was evaluated in vitro.

Although it has been demonstrated in previous studies that HMGB1 might be a potential biomarker for disease activity in SLE, little is known about the clinical implications of auto-antibodies against HMGB1. Therefore, as described in chapter 4, a cross-sectional study was performed to determine anti-HMGB1 antibody levels in patients with SLE and to evaluate if these levels correlated with relevant clinical parameters.
As HMGB1 levels are increased in SLE patients, and HMGB1 possibly contributes to the inflammatory response in SLE, HMGB1 might represent a potential therapeutic target. In chapter 5, the different strategies of HMGB1 inhibition in (auto)immune mouse models are discussed. Moreover, in chapter 6, we investigated whether blocking of HMGB1 with a neutralizing monoclonal antibody ameliorates disease progression in MRL/lpr mice. These mice carry the lymphoproliferation (lpr) mutation and develop a spontaneous autoimmune disease with similarities to human SLE. The mutation is present in the molecule First Apoptosis Signal (Fas, CD95), which is a cell surface-expressed receptor belonging to the TNF-receptor family that induces apoptosis upon interacting with its ligand. On the MRL (MRL/lpr) background this mutation leads to extensive lymphoproliferation and the generation of autoreactive T cells (33-35). As this is a spontaneous model of the disease, the disease course is heterogeneous, affecting mostly female mice.

Involvement of interleukin-17A (IL-17A) has been suggested in SLE. Since the IL-17 receptor A is part of the signaling pathway of many IL-17 family members we investigated the role of IL-17 receptor signaling in development of disease in mice on a B6/lpr background in chapter 7. C57BL/6-lpr (B6/lpr) mice were studied as an animal model of SLE representing a model with a milder disease phenotype than MRL/lpr mice with the first symptoms occurring around 16-20 weeks of age (36). As a relation between HMGB1 and Th17 cells has been reported, HMGB1 and anti-HMGB1 levels were also investigated. Finally, in chapter 8 the results of this thesis are summarized and discussed, and suggestions for future research are proposed.

REFERENCES


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Mobility group box 1 (HMGB1) and anti-HMGB1 antibodies and their relation to disease characteristics in systemic lupus erythematosus. 13: R71.


